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New insights into anti-TNF treatment of ankylosing spondylitis

Mirjam Kirsten de Vries

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VRIJE UNIVERSITEIT

**New insights into anti-TNF treatment of
ankylosing spondylitis**

ACADEMISCH PROEFSCHRIFT

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de Vrije Universiteit Amsterdam,
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promotor: prof.dr. B.A.C. Dijkmans
copromotor: dr. I.E. van der Horst-Bruinsma

*An idea that is developed and put into action
is more important than an idea that exists only as an idea*

Boeddha

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1

General Introduction

GENERAL INTRODUCTION

Ankylosing spondylitis (AS) is a chronic rheumatic disease that affects over 40,000 patients in the Netherlands and has a large impact on physical functioning. Amsterdam has a long tradition in the treatment of AS patients, especially at Reade, the former Jan van Breemen Institute, and at VU University Medical Center (VUmc). VUmc was one of the first centers in the world where AS patients were being treated with infliximab, a new medicine based on the blockade of the pro-inflammatory protein TNF. The results were amazing. Patients who did not have any other treatment options before - except for NSAIDs and physical therapy - experienced a big improvement in pain, stiffness and physical functions. Other TNF blocking agents followed with similar impressive responsiveness. The cohort studies of patients, treated with these agents, provide material for many research projects as described in this thesis.

Characteristics

AS is a chronic inflammatory disease that mainly involves spine and sacroiliac joints. The disease often presents itself with pain in the buttock area (due to inflammation of sacroiliac joints), back pain during the night and morning stiffness. This morning stiffness will last at least one hour, but often many hours, and it will improve with exercises, but it is not relieved by rest. Besides spinal symptoms and joint inflammation, other organs may be affected. AS belongs to a group of diseases which are defined as spondylarthropathies (SpA). SpA consist of common inflammatory rheumatic disorders, including psoriatic arthritis, SpA in patients with inflammatory bowel disease (IBD, such as Crohn's disease (CD) or ulcerative colitis (UC)), reactive arthritis, juvenile SpA and undifferentiated SpA, next to AS. SpA has been classified according to

the European spondylarthropathy study group (ESSG), which includes the presence of inflammatory spinal pain or synovitis (asymmetrical or predominantly in the lower limbs) and one or more of the following: positive family history, psoriasis, inflammatory bowel disease, alternate buttock pain, enthesopathy or sacroiliitis(1). Nowadays, the nomenclature of SpA has changed into axial and peripheral SpA(2). Axial SpA comprises AS and non radiographic axial SpA without radiographic signs of sacroiliitis. Peripheral SpA is dominated by peripheral arthritis with little axial involvement(3). The nomenclature of SpA has changed after the data collection of this thesis and therefore the main focus of research is on AS patients and the nomenclature of axial SpA is not used.

The diagnosis of definite AS has to comply with the 1984 modified New York criteria: obligatory signs are signs of bilateral sacroiliitis grade 2-4 or unilateral sacroiliitis grade 3-4 (visible on the X-ray of the pelvis, Figure 1) plus at least one of the three of the following criteria: inflammatory back pain, limited lumbar spinal motion in sagittal and frontal planes, and decreased chest expansion(4). The clinical manifestations can be divided into spinal and extraspinal features. Extraspinal manifestations are peripheral arthritis, enthesitis, uveitis, psoriasis, inflammatory bowel disease, cardiac involvement (such as aortic root and valve regurgitation, heart conduction disturbances and increased cardiovascular risk) pulmonary involvement (pulmonary fibrosis, bronchiectasis) or, occasionally, amyloidosis.

Epidemiology

The prevalence of AS ranges between 0.1-1.4% and the male-to-female ratio is approximately 3:1(5;6). The majority of the patients have their first symptoms between the ages of 15 and 40(7). In most cases, however, there is a significant delay in diagnosis with an average delay in Western-Europe of 7.5 years(8).

This delay in diagnosis might be explained by the nonspecific, insidious symptoms of low back pain at the onset of the disease.

Figure 1: Sacroiliitis (grade 2, bilateral).



Pathogenesis

The cause of AS is multifactorial, consisting of both hereditary and environmental factors, which have not all been elucidated yet. With respect to hereditary factors it is known that a strong genetic predisposition can be ascribed to the human leukocyte antigen B27 (HLA-B27), which is prevalent in more than 95% of the AS patients. This antigen is also present in 8% of the healthy Dutch Caucasians(9), but is has higher prevalences in the northern parts of Scandinavia (16%)(10).

Furthermore, it has been postulated that certain bacterial infections, such as Chlamydia(11) or gastrointestinal infections (with Salmonella, Shigella, Yersinia or Campylobacter) may contribute to the development of AS, as similarly described in the onset of reactive arthritis.

There is an increased concentration of T cells, macrophages and pro-inflammatory cytokines in AS(12).

The course of the disease

The course of AS varies between mild, with little functional disability, and a very severe, disabling form in a minority of cases. Rudwaleit et al identified several prognostic factors for severe disease, such as male sex and elevated CRP(13).

Spinal inflammation can result in formation of syndesmophytes of the spine that may lead to ankylosis (Figure 2) and thoracic kyphosis. The same process of inflammation with bone repair resulting in ankylosis occurs in the sacroiliac joints (grade 4) of most AS patients. Furthermore, extraspinal complications may occur. Peripheral arthritis occurs in approximately one third of the patients, predominantly in the knees, hips and shoulders and usually in an asymmetrical pattern(14). Besides impairment of function, particularly at an early stage of the disease, arthritis results in cartilage degeneration and it might necessitate joint replacement procedures. Inflammation at the insertions of ligaments, tendons, or joint capsules to bone, which is termed enthesitis, is a characteristic feature of spondyloarthropathy(15).

Vertebral fractures, in particular of the cervical spine, and cervical spine dislocations can cause neurological deficits after minor trauma as the spine is fragile due to ankylosis and osteoporosis. This complication has a Standard Morbidity Ratio of 7.6(16-18) and can be overlooked in daily clinical practice due to an overlap of symptoms (back pain) and difficulties in radiographic detection due to additional bone formation of the spine.

Acute anterior uveitis occurs in 25-30% of the patients and can be the presenting symptom of the disease. It is characterised by a red eye, unilateral ocular pain and photophobia.

About 5-10% of the AS patients have diarrhoea due to concurrent inflammatory bowel disease (IBD), either Crohn's disease or ulcerative colitis(15;19-21).

Furthermore, AS is associated with well known characteristic cardiovascular manifestations, particularly conduction disturbances and aortic insufficiency (22). Notably, there are some suggestions for a higher prevalence of left ventricular dysfunction and the risk of cardiovascular events is doubled in AS population(23).

Figure 2: Complete ankylosis of the cervical spine.



Therapy

Non-pharmacological treatment of AS includes patient information, physical therapy and stimulating the patient to exercise frequently. Non steroidal anti-inflammatory drugs (NSAIDs), such as diclofenac or naproxen, are recommended as first line medical treatment and they generally decrease pain and morning stiffness. Several studies have shown that selective COX-2 inhibitors, such as etoricoxib and celecoxib, are very effective in AS as well(24;25). The selective COX-2 inhibitors can be used in case of relative contraindications for NSAID use, such as dyspepsia or IBD.

Unfortunately, most disease modifying anti-rheumatic drugs (DMARDs) do not seem to be effective in AS, although properly conducted studies with high dosages are often lacking(26). Sulphasalazine may be considered for AS patients with peripheral arthritis(27) and there is some debate about whether it may be effective for spinal complaints as well(28;29).

Therapy with biologicals, especially tumor necrosis factor (TNF) inhibitors, have transformed the treatment paradigm in AS. These drugs are produced with recombinant DNA technologies and they block the protein TNF, which is one of the main proinflammatory cytokines.

At the time of writing this thesis, three TNF blocking agents were approved and registered for use in AS after large multicenter randomised clinical trials had been performed. The first is infliximab, a chimeric monoclonal anti-TNF antibody of human IgG1(30), the second, etanercept, a fusion protein consisting of two TNF receptors linked to the Fc region of human IgG1(31), and the third, adalimumab, a humanized monoclonal anti-TNF antibody of human IgG1(32). Treatment of AS with anti-TNF has proven to be safe and very effective with response rates varying from 50% to 76%, which are similar for all three TNF blocking agents(30-32). These drugs differ in the way of administration.

Infliximab is administered intravenously in 6-8 weeks intervals and etanercept and adalimumab are given as subcutaneous injections once a week or every other week. Another difference is the efficacy on extra articular manifestations. In contrast to infliximab and adalimumab, etanercept is not effective IBD(33). The efficacy of etanercept on the recurrence of attacks of uveitis seems to be lower than that of the other TNF blocking agents but this needs some further research(32;34;35). When most studies described in this thesis were finished, other TNF blocking agents emerged such as golimumab, which has recently been registered and reimbursed by health insurance for the treatment of AS in the Netherlands. Phase III trials are currently being conducted with Certolizumab pegol, a polyethylene glycol (PEG) linked Fab' antibody fragment of a humanized TNF inhibitor monoclonal antibody(36) in AS.

Increased susceptibility to infections, specifically reactivation of latent tuberculosis is the most frequent side-effect of TNF blocking agents(37). Therefore, every patient is screened for latent tuberculosis before start of treatment according to the Dutch consensus guideline, available at www.nvr.nl. A complicated side-effect of biologicals is their ability to provoke an immune response, triggered by foreign components of the protein(38). Additionally, auto-antibodies can be formed(39).

The high costs of TNF blocking agents (approximately 15.000 €/year)(40) necessitate the presence of assessment tools in order to determine the disease activity and response to therapy.

Disease activity scores and functional assessments

Unlike other rheumatic diseases, such as rheumatoid arthritis, acute phase reactants such as erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) do not always reflect disease activity in every AS patient(41). Therefore, other outcome and disease activity parameters have been developed and

validated over the past years. Up till now, disease activity is mostly determined with the Bath ankylosing spondylitis disease activity index (BASDAI)(42). This is a self-administered questionnaire consisting of 6 questions (Appendix). The BASDAI is used for determination of ASAS response. For example, a 20% decrease of the BASDAI or decrease with 2 units on a scale from 0 to 10 is fulfilment of ASAS20 response.

Physical function can be measured with the Bath ankylosing spondylitis functional index (BASFI), spinal mobility with the Bath ankylosing spondylitis metrology index (BASMI) and questionnaires (visual analogue scales and numeric rating scales) have been developed to assess pain and global disease activity (Appendix).

Recently, the Assessment of SpondyloArthritis international Society (ASAS) has developed a new tool to assess disease activity: the AS disease activity score, the ASDAS(43). It combines five disease activity variables with only partial overlap, resulting in one single score with better truth (validity), enhanced discriminative capacity and improved sensitivity to change as compared to single-item variables(43;44). The ASDAS was not used as a disease activity parameter in our studies because at the time the ASAS20 improvement criterion was used in most clinical trials. The definition of this response parameter is described in Table 1.

Table 1: ASAS20, ASAS response and the two ASDAS formulas: ASDAS-CRP (preferred) and ASDAS-ESR (alternative).

ASAS20	<p>An improvement of $\geq 20\%$ and an absolute improvement of ≥ 1 units on a 0-10 scale in ≥ 3 domains of the following 4 domains.</p> <ul style="list-style-type: none"> -Patient global assessment -Pain (the average of VAS total and nocturnal pain scores) -Function (BASFI) -Inflammation <p>Absence of $\geq 20\%$ deterioration in the remaining domain.</p>
ASAS response	Improvement of the BASDAI with 50% or an absolute improvement of 2 points on a 0-10 scale.
ASDAS-CRP	$0.12 \times \text{Back Pain} + 0.06 \times \text{Duration of Morning Stiffness} + 0.11 \times \text{Patient Global} + 0.07 \times \text{Peripheral Pain/Swelling} + 0.58 \times \text{Ln}(\text{CRP}+1)$
ASDAS-ESR	$0.08 \times \text{Back Pain} + 0.07 \times \text{Duration of Morning Stiffness} + 0.11 \times \text{Patient Global} + 0.09 \times \text{Peripheral Pain/Swelling} + 0.29 \times \sqrt{\text{ESR}}$
<p>ASDAS, ankylosing spondylitis disease activity score; $\sqrt{\text{ESR}}$, square root of the erythrocyte sedimentation rate (mm/h); $\text{Ln}(\text{CRP}+1)$, natural logarithm of the C-reactive protein (mg/L) + 1.</p> <p>Back pain, patient global, duration of morning stiffness and peripheral pain/swelling are all assessed on a visual analogue scale (from 0 to 10cm) or on a numerical rating scale (from 0 to 10).</p> <p>Inflammation: the average of question 5 and 6 of the BASDAI's last two VAS concerning morning stiffness intensity and duration.</p> <p>Back pain, BASDAI question 2: "How would you describe the overall level of AS neck, back or hip pain you have had?".</p> <p>Duration of morning stiffness, BASDAI question 6: "How long does your morning stiffness last from the time you wake up?".</p> <p>Patient global: "How active was your spondylitis on average during the last week?"</p> <p>Peripheral pain/swelling, BASDAI question 3: "How would you describe the overall level of pain/swelling in joints other than neck, back or hips you have had?"</p>	

Thesis outline

Many AS patients (around 60%) show a good response to treatment with TNF blocking agents. In a minority however, these drugs do not show efficacy (primarily) or they lose their effectiveness during treatment (secondarily). In some cases allergic reactions occur. This topic of unresponsiveness was explored in **Section I (Chapter 2-4)**. The main issue was whether non-response and allergic reactions are related to immunogenicity of TNF blocking agents in AS.

In order to select eligible patients for treatment with anti-TNF, patients were categorized in according to their disease activity score, the BASDAI. As the BASDAI is a patient-reported measure, and objectiveness might therefore be disputable, there is some doubt about the reliability of this tool. In **Section II** the changes of other biomarkers of disease activity were studied in relation to the BASDAI and changes after treatment with anti-TNF were determined. In **Chapter 5.1** three types of inflammatory markers (ESR, CRP and serum amyloid A protein: SAA) and their change upon TNF-blockade were investigated. CRP levels are used more frequently to determine disease activity in AS patients. These levels, though, do not necessarily reflect disease activity in each patient. In **Chapter 5.2**, the relation between CRP levels and common single-nucleotide polymorphisms (SNPs) and haplotypes in the CRP gene were studied. Additionally, the relation between CRP levels and the BASDAI were investigated.

Chapter 6 contains a review on Andersson lesions (ALs), destructive vertebral lesions leading to pseudarthrosis, which can be a severe complication in long standing AS. The prevalence of these lesions was studied in our cohort of AS patients, detected with MRI.

Inflammation also plays an important role in atherosclerosis(36;45-47). Therefore, a study on dyslipidemia and its relation with the inflammatory markers was depicted in **Chapter 7**. In particular, the effect of anti-TNF on the composition of HDL particles was studied.

In **Section III** we focus on extraspinal manifestations. At VUmc, a number of patients who suffered from both AS and IBD are treated. Therefore, we were interested in the serological similarities between AS and IBD patients, and whether serological markers could be detected in AS patients who might be prone to develop IBD. This study was depicted in **Chapter 8**. Finally, in **Chapter 9**, cardiac manifestations of AS are addressed and the relation between AS-related characteristics and conduction disturbances was investigated.

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Section I

Immunogenicity

2.1

Inefficacy of infliximab in ankylosing spondylitis is correlated with antibody formation.

Mirjam K. de Vries¹, Gerrit Jan Wolbink^{2,3}, Steven O. Stapel², Els R. de Groot², Ben A. C. Dijkmans^{1,3}, Lucien A. Aarden², Irene E. van der Horst-Bruinsma¹.

Rheumatology Department VU University Medical Center¹, Sanquin Research², Rheumatology Department Jan van Breemen Institute/Reade³, Amsterdam, the Netherlands.

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Tumor necrosis factor blocking agents such as infliximab have proved to be effective in patients with ankylosing spondylitis (AS) as up to 60-70% of the patients meet the 20% response criteria of assessment in ankylosing spondylitis (ASAS-20)(1;2). However, it cannot be explained why 30% of patients fail to respond and develop adverse reactions.

In rheumatoid arthritis, inefficacy to infliximab was associated with low serum trough infliximab levels and the presence of antibodies to infliximab(3).

This study was designed to identify whether infliximab levels and antibodies to infliximab predict clinical inefficacy and adverse events in AS.

Eight patients with active AS (fulfilling the 1984 modified New York Criteria(4)) were treated according to the international ASAS consensus statement(5), with infliximab 5mg/kg given intravenously at baseline, week 2, 6, and 12, and every 6 weeks thereafter. Sera were collected at 12 and 24 weeks before infusing.

At every visit, questionnaires (e.g., Bath ankylosing spondylitis disease activity index) to assess ASAS-20 were obtained and routine laboratory tests were performed. These data were correlated with disease activity (ASAS-20), serum trough infliximab levels and antibody levels.

All patients were men, with a median (range) age of 47 (24-52) years, and were human leukocyte antigen B27 (HLA-B27) positive, with a median (range) disease duration of 11 (1-28 years) years (Table 1). Patient 1 was concomitantly treated with 15 mg methotrexate weekly and patient 3 was treated with cyclosporine and sulfasalazine.

Most patients responded well to infliximab with a considerable decline in Bath ankylosing spondylitis disease activity index (BASDAI), erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP), high serum trough levels of infliximab and no development of antibodies to infliximab. However,

two non-responders did not show detectable serum trough infliximab levels and developed antibodies to infliximab after, respectively, 12 and 24 weeks. Patient 3 did not respond to treatment at all, whereas patient 5 met the ASAS-20 response criteria but had an increase in ESR and CRP levels. Both patients developed an infusion reaction to infliximab.

Table 1: Clinical response to infliximab in ankylosing spondylitis patients in relation to infliximab levels and antibodies to infliximab after 24 weeks.

<i>Patient</i>	<i>BASDAI</i> <i>Week 0</i> <i>Mean:5.5</i> <i>Median:5.2</i>	<i>BASDAI</i> <i>Week 24</i> <i>Mean:1.9</i> <i>Median:1.8</i>	<i>ESR</i> <i>Week 0</i> <i>Mean: 43</i> <i>Median:6.5</i>	<i>ESR</i> <i>Week 24</i> <i>Mean: 11</i> <i>Median:8.5</i>	<i>CRP</i> <i>Week 0</i> <i>Mean: 52</i> <i>Median:25</i>	<i>CRP</i> <i>Week 24</i> <i>Mean: 8</i> <i>Median:5</i>	<i>ASAS-</i> <i>20</i>	<i>Infliximab</i> <i>level</i> <i>(ng/ml)</i>	<i>antibodies</i> <i>to</i> <i>infliximab</i> <i>(ng/ml)</i>
1	6.4	1.2	88	4	115	4	+	17800	0
2	4.5	0.7	90	8	120	6	+	10100	0
**3	*	*	22	26	14	21	*	0	7200
4	7.2	0.0	72	18	104	6	+	20600	0
**5	4.7	3.1	12	18	7	20	+	0	15600
6	4.5	1.8	23	9	11	<2.5	+	16000	0
7	5.2	4.1	10	6	7	<2.5	+	10300	0
8	6.3	2.1	30	1	36	<2.5	+	16400	0

BASDAI, Bath ankylosing spondylitis disease activity index (scale 0-10); ESR, erythrocyte sedimentation rate (mm/hour); CRP, C-reactive protein (mg/l); ASAS-20 response.

* not done due to severe visual impairment.

** are considered as non-responders due to increase of inflammatory parameters.

In this study on eight patients with AS, a correlation between efficacy of infliximab and high levels of serum trough infliximab was shown. In 25% of these patients with AS antibodies to infliximab developed within 24 weeks in association with undetectable serum trough infliximab levels, inefficacy of infliximab and infusion reactions.

The number of patients, however, is too small to draw definite conclusions, but interestingly, these data point in the same direction as described previously in rheumatoid arthritis(3). Lower serum trough infliximab levels could be

explained by enhanced clearance because of immune complex formation between anti-infliximab antibodies and infliximab. To prevent antibodies to infliximab formation that might inhibit efficacy of infliximab, it might be helpful to increase the dosage of infliximab (as occurs in treatment of rheumatoid arthritis with infliximab), to shorten the interval between infliximab infusions (as is currently the strategy in Crohn's disease) or to provide coadministration of other immunosuppressives (such as methotrexate). These data should be confirmed in a larger group of patients with AS to develop a more patient-specific treatment, which might predict the inefficacy of infliximab at an early stage and might prevent adverse reactions.

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2.2

Decreased clinical response to infliximab in ankylosing spondylitis is correlated with anti-infliximab formation.

Mirjam K. de Vries¹, Gert Jan Wolbink², Steven O. Stapel², Henk de Vrieze², J. Christiaan van Denderen³, Ben A. C. Dijkmans¹, Lucien A. Aarden², Irene E. van der Horst-Bruinsma¹.

Rheumatology Department VU University Medical Center¹, Sanquin Research², Rheumatology Department Jan van Breemen Institute/Reade³, Amsterdam, the Netherlands.

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ABSTRACT

Objectives: Correlation of serum trough infliximab levels and antibodies to infliximab (anti-infliximab) with clinical response in ankylosing spondylitis (AS).

Methods: In accordance with the international assessments in ankylosing spondylitis (ASAS) consensus statement, patients were treated with infliximab (5 mg/kg) every 6 weeks after a starting regimen. Preinfusion sera were collected at baseline, 24 and 54 weeks.

At every visit, the 20% improvement response (ASAS-20) was assessed and laboratory tests performed.

Results: 24 of the 38 (63%) patients fulfilled ASAS-20 response criteria after 24 weeks of treatment and 21 (53%) after 54 weeks. After 54 weeks, 11 (29%) patients showed undetectable serum trough infliximab levels and detectable anti-infliximab; six of these patients developed an infusion reaction. Anti-infliximab was found significantly more often ($p=0.04$) in ASAS-20 non-responders compared with responders at week 54. Serum trough infliximab levels were significantly ($p<0.0001$) lower in patients with (mean: 0.02 mg/l) than in those without (mean: 12.7 mg/l) anti-infliximab.

Conclusions: In AS, high levels of serum trough infliximab correlated with a good clinical response. Detection of anti-infliximab within 54 weeks is associated with undetectable serum trough infliximab levels, reduced response to treatment and increased risk of developing an infusion reaction.

INTRODUCTION

Large randomised clinical trials have shown that tumor necrosis factor blocking agents such as infliximab are very effective in ankylosing spondylitis (AS)(1). It is unknown why more than 30% of patients with AS fail to respond, or why some initial responders lose responsiveness during treatment and in some cases even develop an infusion reaction. The non-responsiveness to infliximab might be due to the development of antibodies against it, which has been described in patients with rheumatoid arthritis and Crohn's disease(2-5).

In AS, we recently showed in a small group of patients that detection of anti-infliximab was associated with undetectable serum trough infliximab levels, a reduced response to treatment and a higher risk of infusion reactions(6).

The aim of this study was to evaluate these data in a larger group of patients with AS who were treated for a longer period of time and to specify the influence on infliximab levels.

METHODS

All consecutive patients with AS (according to the 1984 modified New York Criteria(7)) who received treatment with infliximab in our center were included in this study.

Disease activity was measured with the Bath ankylosing spondylitis disease activity index (BASDAI)(8) and the assessments in ankylosing spondylitis 20% response criteria (ASAS-20)(9). Active disease was defined as a BASDAI \geq 4. Response to treatment with infliximab was defined as fulfilment of the ASAS-20 response criteria.

Patients with AS were treated with intravenous infliximab, 5 mg/kg bodyweight at baseline, weeks 2 and 6, and every 6 weeks thereafter. This treatment was initiated in accordance with the international ASAS consensus statement(9). In

case of decrease of clinical response, the dose of infliximab was increased to 7.5 mg/kg. At each visit the presence of infections, side-effects or infusion reactions, and the cause for discontinuation of therapy were recorded.

Questionnaires and routine laboratory tests were obtained. Preinfusion sera were collected at baseline, weeks 24 and 54, before any dose escalation and at two consecutive visits after dose escalation. After 24 weeks of treatment, serum samples were collected from 15 patients to measure infliximab levels 2 weeks after the infliximab infusion.

Validated immunoassays (Sanquin Research, Amsterdam, the Netherlands) were used for detection of anti-infliximab and serum trough infliximab levels(5). Trough serum infliximab levels were measured by ELISA, based on the principle that infliximab is captured through its ability to bind tumor necrosis factor. The assay, which was described previously, was modified recently. It currently uses specific polyclonal rabbit antibodies to infliximab for detection instead of the monoclonal anti human IgG that was previously used. The sensitivity of detection is 0.0003 mg/l.

A radioimmunoassay (RIA) was used for anti-infliximab detection(5). Arbitrary units per ml (AU/ml) were expressed as absolute amounts of infliximab-specific IgG (mg/l)(10). (1 AU = 12 ng of infliximab-specific IgG). The cutoff value for IgG anti-infliximab was determined by assaying in our anti-infliximab test 100 plasma samples from blood donors sent to Sanquin for IgG anti-tetanus toxoid testing. The average result (AU/ml) + 6 SD was 12 AU/ml (0.144 mg/l).

The clinical data and presence of human leukocyte antigen B27 (HLA-B27) were used to correlate disease activity with serum trough infliximab levels and anti-infliximab levels. Differences between groups were tested with the Mann-Whitney U test. Associations were calculated with logistic regression. The threshold for significance was set at $p < 0.05$. The last observation was carried forward for patients who dropped out before week 54 .

RESULTS

Demographic and clinical characteristics of the 38 patients included are shown in Table 1.

Table 1: Demographic and clinical variables at baseline, week 24 and at week 54 of patients with ankylosing spondylitis (N=38).

<i>Variables</i>	<i>Baseline</i>	<i>Week 24</i>	<i>Week 54</i>
Male sex, N (%)	26 (68)		
Age in years	40 (10)		
HLA-B27 positivity, N (%)	32 (84)		
IBD, N (%)	6 (16)		
Use corticosteroids, N (%)	3 (8)		
Use other immunosuppressives, N (%)	6 (16)		
BASDAI	6.4 (1.2)	3.6 (2.6) *	4.1 (3.0) *
Morning stiffness	6.3 (2.2)	3.0 (2.5) *	3.5 (3.2) *
Global disease activity VAS	6.8 (1.3)	4.3 (2.9) *	4.9 (3.4) #
CRP	37 (34.2)	9.3 (10.7) *	15.8 (21.1) ~
Detectable serum trough infliximab, N (%)	0	31 (82)	27 (71)
Anti-infliximab, N (%)	0	7 (18)	11 (29)

*Except when indicated otherwise, the values are the mean (SD).

HLA-B27, human leukocyte antigen B27; IBD, inflammatory bowel disease; BASDAI, Bath ankylosing spondylitis disease activity index (0-10 cm); Morning stiffness, mean of item 5+6 of BASDAI (0-10 cm); Global disease activity Visual Analogue Scale (0-10 cm); CRP, C-reactive protein, normal <8.0 mg/l; detectable serum trough infliximab (% detectable); anti-infliximab, antibodies to infliximab (% detectable).

Compared with baseline *: p<0.001; #: p=0.005; ~: p=0.003

Four patients were lost to follow-up before week 54: one wanted to become pregnant, one preferred to be treated in a hospital nearby and two because of comorbidities.

There was a significant decrease in BASDAI , morning stiffness, global disease activity and C-reactive protein after 24 and 54 weeks of treatment (Table 1) and all pre-treatment samples showed undetectable infliximab levels and no anti-infliximab. We did not detect anti-infliximab in the presence of infliximab.

After 24 weeks, 24 patients (63%) met ASAS-20 response criteria. Responders showed higher mean serum trough infliximab levels, and only two patients (8%) showed anti-infliximab, compared with 5 (36%) of the non-responders ($p=0.08$).

After 54 weeks of treatment, ASAS-20 response criteria were met by 21 patients (53%). The mean serum trough infliximab level for responders was significantly ($p<0.01$) higher than that of the non-responders (8.2 mg/l vs 6.3 mg/l; Figure 1) and anti-infliximab was significantly ($p<0.04$) more often found in non-responders. Only 5% (1 of 21) of the responders showed anti-infliximab, compared with 59% (10 of 17) of the non-responders. In total, 9% (1 of 11) patients with detectable anti-infliximab was classified as a responder at week 54, compared with 74% (20 of 27) of patients without anti-infliximab (Figure 2).

Figure 1: Serum trough infliximab level for responders (N=21; 8.2 mg/l) and non-responders (N=17; 6.3 mg/l) according to the ASAS-20 response criteria, at week 54.

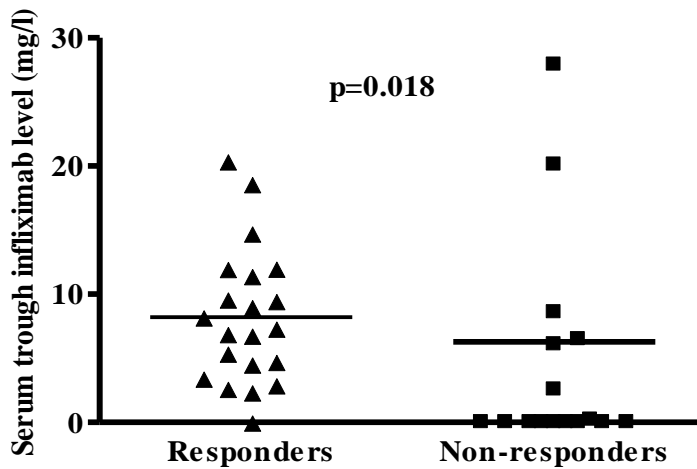
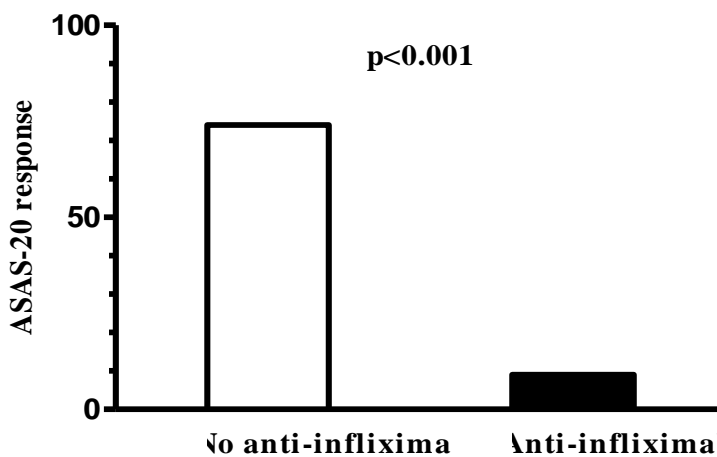


Figure 2: Percentage of patients (N=38) with (9%) and without (74%) anti-infliximab fulfilling the ASAS-20 response criteria at week 54.



After correction for probable confounding variables such as sex and HLA-B27, the absence of anti-infliximab remained a significant determinant for ASAS-20 response with an odds ratio (OR) of 100 (95% CI 5.2 to 1000). Remarkably, the presence of anti-infliximab was significantly associated with the absence of HLA-B27 (OR=7.1; 95% CI 1.1 to 47.6; Pearson χ^2 , $p=0.03$).

Two weeks after the infusion of week 24, significantly lower infliximab levels were measured (20 mg/l compared with 51 mg/l; $p<0.01$) in patients who developed anti-infliximab within 54 weeks of treatment.

In 12 patients, dose was increased within the 54 weeks, because of insufficient clinical response. Nine (75%) of these patients showed anti-infliximab antibodies. Increase in dose did not result in a significant increase of the serum trough infliximab level ($p=0.33$), or a significant decrease in the anti-infliximab level ($p=0.90$) and BASDAI ($p=0.39$). However, 2 of 12 patients reported longer duration of effect.

Infusion reactions occurred in six patients. Most reactions were mild, and all patients recovered after supportive therapy. Treatment with infliximab was stopped in each case. Every infusion reaction was preceded by development of anti-infliximab and consequently undetectable serum trough infliximab levels. All antibodies to infliximab consisted of IgG1 and IgG4 subtypes. Although these infusion reactions resemble a type 1 allergic reaction, no IgE was detected. One patient's pre-infusion serum contained an anti-infliximab level of 6.4 g/l, indicating that approximately half of his total serum IgG consisted of infliximab-specific antibodies.

DISCUSSION

A good clinical response of AS to treatment with infliximab was correlated with the presence of high serum trough infliximab levels and the absence of anti-infliximab antibodies, and inefficacy with the reverse. Moreover, these data demonstrate that anti-infliximab antibodies precede an infusion reaction.

The mechanism of the decrease in efficacy can be explained by the lower serum trough infliximab levels, probably caused by enhanced clearance due to immune complex formation of anti-infliximab antibodies and infliximab. A recent study in RA showed an enhanced clearance as a consequence of this process and an accumulation in the macrophage-phagocyte system (liver and spleen)(11).

Indeed, in those patients with AS who developed detectable anti-infliximab within 54 weeks of treatment with infliximab, a significantly lower infliximab level was found 2 weeks after infusion compared with patients who did not develop anti-infliximab.

Often, the infliximab dose is increased in AS when responsiveness decreases, but reasons for dose escalation in AS are not yet well defined. In our small sample, no clear increase in serum trough infliximab level after dose escalation was shown.

Another option is to try to prevent anti-infliximab formation with the concomitant administration of other immunosuppressive drugs such as methotrexate; however, this medication is not efficacious in AS(12).

Remarkably, absence of HLA-27 shows significant correlation with anti-infliximab formation. Further genetic evaluation will be performed to unravel this interesting observation.

It also has to be investigated whether coadministration of immunosuppressive drugs inhibits anti-infliximab formation, and whether infliximab levels can be used for determination of the optimum dose of infliximab in AS.

In accordance with our previous report, the efficacy of infliximab in AS is clearly related to infliximab levels and the formation of anti-infliximab antibodies. Detection of anti-infliximab antibodies within 54 weeks is associated with undetectable serum trough infliximab levels, reduced response to treatment and increased risk of development of an infusion reaction.

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3.1

Decreased clinical response to adalimumab in ankylosing spondylitis is associated with antibody formation.

Mirjam K. de Vries¹, Elisabeth Brouwer⁵, Irene E. van der Horst-Bruinsma¹, Anneke Spoorenberg⁶, J. Christiaan van Denderen³, Anna Jamnitski³, Michael T. Nurmohamed^{1,3}, Ben A. C. Dijkmans^{1,3}, Lucien A. Aarden^{2,4}, Gert Jan Wolbink^{2,3}.

Rheumatology Department VU University Medical Center¹, Sanquin Research², Rheumatology Department Jan van Breemen Institute/Reade³, Landsteiner laboratory Academic Medical Center⁴, Amsterdam, Rheumatology Department University Medical Center Groningen⁵, Rheumatology Department Medical Center Leeuwarden⁶, the Netherlands.

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Treatment with anti-tumor necrosis factor (TNF) is very effective in most patients with ankylosing spondylitis (AS), but inefficacy occurs in about 40% of cases(1). Antibody formation against TNF blocking agents is an increasingly recognised problem(2), however, no data have yet been reported on antibody formation against adalimumab (anti-adalimumab) in AS. Lack of response can be explained in two ways. Firstly, TNF might not be important for disease activity in certain patients; and secondly, TNF inhibition might be insufficient. The latter could be caused by excessive production of TNF, low compliance of the patient, insufficient dosing or an enhanced clearance of adalimumab due to antibody formation. Adalimumab is a fully human monoclonal antibody against TNF but, despite this fact, an immune response still can be provoked by the antigen binding site also known as the idiotype. In previous studies we have described the problem of immunogenicity of TNF blocking drugs in patients with rheumatoid arthritis (RA)(3), in patients with AS treated with infliximab(4) and in patients with RA treated with adalimumab(5), and concluded that the presence of antibodies against infliximab or adalimumab was associated with low or undetectable serum levels of infliximab or adalimumab and clinical non-response.

The objective of the present study was to investigate the relation between the formation of anti-adalimumab, serum adalimumab levels and clinical response in AS.

Patients with AS(6) were treated with adalimumab, 40 mg every other week, according to the international ASAS consensus statement(7;8). Clinical response was defined as a 50% improvement or an absolute improvement of 2 points on the BASDAI scale (0-10). Serum samples were collected at baseline and after 3 and 6 months of treatment. Serum adalimumab levels were determined with an ELISA and anti-adalimumab was measured with a validated antigen binding test. The assays used were similar to those described previously

for the detection of infliximab levels and antibodies against infliximab(4;5).

Thirty-five patients were included. After 6 months of treatment, 18 were ASAS responders (Table 1). Within 6 months of treatment, 11 patients developed anti-adalimumab with low or undetectable adalimumab levels, 9 were ASAS non-responders ($p=0.012$) and 1 had an allergic reaction with flushing, dyspnoea and undetectable serum adalimumab levels (Figure 1).

Table 1: Baseline characteristics and clinical variables.

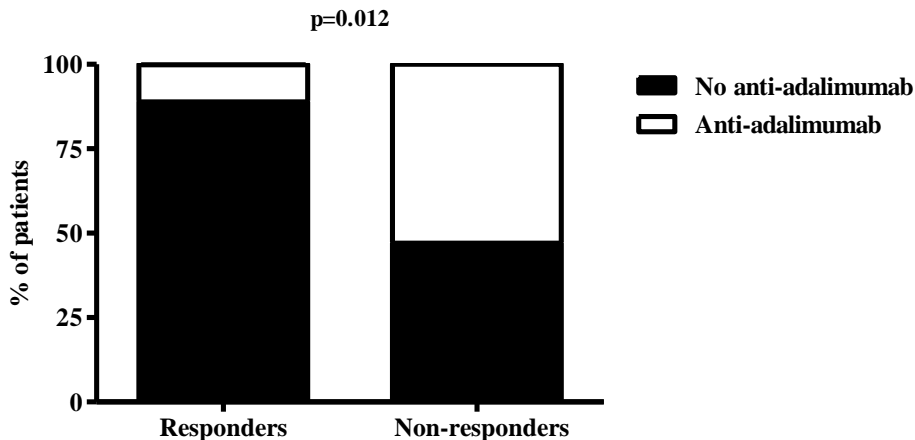
	<i>Baseline</i> (<i>N=35</i>)	<i>t=6 months</i>
Male sex, N (%)	27 (76)	
Mean (SD) age (years)	43 (12)	
Disease duration (years)	9 (3.5-16.5)	
HLA-B27 positivity, N (%)	24 (69)	
Presence of IBD, N (%)	4 (12)	
Presence of uveitis, N (%)	15 (46)	
Presence of arthritis, N (%)	11 (32)	
Anti-TNF used before, N (%)	10 (29)	
BASDAI	6.2 (4.9-7.6)	3.0 (1.2-5.3) *
ASAS response (N responders , %)		18 (51%)
Global disease activity	7.2 (5.9-8.0)	2.1 (1.0-5.0) *
ESR	31 (19-44)	7 (3-15)*
CRP	21 (10-35)	<5 (2-6) *

Unless otherwise indicated, values are the median (interquartile range).

ASAS, assessments in ankylosing spondylitis (decrease of 50% or > 2; global disease activity (0-10 scale)); BASDAI, Bath ankylosing spondylitis disease activity index (0-10 scale); CRP, C-reactive protein (normal <10.0 mg/l); ESR, erythrocyte sedimentation rate (normal <15 mm/h); HLA-B27, human leukocyte antigen B27; IBD, inflammatory bowel disease.

* $p<0.0001$ compared with baseline.

Figure 1: Relation between the presence of anti-adalimumab and response of ankylosing spondylitis to treatment with adalimumab.



Thus, anti-adalimumab was detected in 31% of the patients after 6 months of treatment and this corresponded with diminished or undetectable serum adalimumab levels in these patients. These preliminary observations will need confirmation in a larger study. In contrast with the treatment of RA, adalimumab is given without methotrexate in the treatment of AS. This might be an explanation for the higher incidence of anti-adalimumab formation in AS. In Crohn's disease and in RA, the concomitant use of immunosuppressive drugs or corticosteroids has been proved to decrease antibody formation against infliximab(9;10).

To date, no other papers have reported on immunogenicity in the treatment of AS with adalimumab and no systemic allergic reactions have been described. The detection of antibodies might predict the inefficacy of adalimumab and should be explored further for use in daily clinical practice.

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3.2

Adalimumab in juvenile rheumatoid arthritis. Comment on the article by Lovell and colleagues.

Mirjam K. de Vries¹, Irene. E. van der Horst-Bruinsma¹ and Gerrit Jan Wolbink².

Rheumatology Department VU University Medical Center¹, Sanquin Research²,
Amsterdam, the Netherlands.

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To the editor: Lovell and colleagues conclude that adalimumab appeared to be efficacious in children with polyarticular-course juvenile rheumatoid arthritis. Immunogenicity is emerging as an important problem in treatment with monoclonal antibodies(1). Lovell and colleagues report that approximately 16% of the patients had anti-adalimumab antibodies, which did not seem to interfere with the efficacy of adalimumab. This incidence is higher than the 5% incidence among adults with rheumatoid arthritis, reported by Abbott Laboratories(2). We wonder which method was used to detect these antibodies, since the use of different methods hampers the comparison of results(1). The findings of Lovell and colleagues are in accordance with those of our study, in which anti-adalimumab antibodies were detected in 17% of the patients with rheumatoid arthritis after 6 months of treatment(3). In our study, however, the presence of these antibodies was associated with low or undetectable serum adalimumab levels and a reduced clinical response, which was also observed in Crohn's disease(4). Therefore, it would be interesting to know more details about the relation among anti-adalimumab antibodies, serum adalimumab levels, and the American College of Rheumatology Pediatric response.

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4

Immunogenicity does not influence treatment with etanercept in patients with ankylosing spondylitis.

Mirjam K. de Vries¹, Irene E. van der Horst-Bruinsma¹, Michael T. Nurmohamed^{1,3}, Lucien A. Aarden², Steven O. Stapel², Mike J.L. Peters^{1,3}, J. Christiaan van Denderen³, Ben A.C. Dijkmans¹, Gerrit J. Wolbink^{2,3}.

Rheumatology Department VU University Medical Center¹, Sanquin Research², Rheumatology Department Jan van Breemen Institute/Reade³, Amsterdam, the Netherlands.

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ABSTRACT

Background: Immunogenicity, specifically the onset of antibodies against Tumor Necrosis Factor (TNF) blocking agents, seems to play an important role in non-response to treatment with these drugs.

Objectives: To assess the relation of clinical response of ankylosing spondylitis (AS) to etanercept with etanercept levels, and the presence of antibodies to etanercept.

Methods: AS patients were treated with etanercept 25 mg twice weekly, according to the international ASAS consensus statement. Sera were collected at baseline, after 3 and 6 months of treatment. Clinical response was defined as a 50% improvement or as an absolute improvement of 2 points on a 0-10 scale of BASDAI.

Functional etanercept levels were measured by a newly developed ELISA, measuring the binding of etanercept to TNF. Antibodies against etanercept were measured with a two-site assay and antigen binding test. Clinical data were used to correlate disease activity with serum etanercept levels.

Results: 53 consecutive patients were included. After 3 months of treatment 40 patients (76%) fulfilled the response criteria. Mean etanercept levels were 2.7 mg/l and 3.0 mg/l after 3 and 6 months respectively. Characteristics and etanercept levels of responders and non-responders were similar. No antibodies to etanercept were detected with any of the assays.

Conclusion: Etanercept levels of responders and non-responders were similar and no antibodies to etanercept were detected with any of the assays. This study indicates that etanercept is much less immunogenic compared with the other TNF blocking agents.

INTRODUCTION

Ankylosing spondylitis (AS) is a chronic inflammatory disease, which can result in invalidating deformities of the joints and spine at an early age. Until recently, treatment was mainly based on non-steroidal anti-inflammatory drugs (NSAIDs) and physical therapy. Most disease modifying anti-rheumatic drugs (DMARDs) do not seem to be effective in AS, although properly conducted studies are lacking(1). The introduction of tumor necrosis factor (TNF) blocking agents, i.e., infliximab(2), etanercept(3) and adalimumab(4) have changed the treatment options in AS radically. The majority of AS patients, who fulfil the assessment of ankylosing spondylitis (ASAS) guidelines for anti-TNF treatment, respond very well. Nevertheless, TNF blocking agents still fail to reach efficacy in approximately 30% of patients with AS.

A possible explanation for this failure could be the formation of antibodies, which results in lower or undetectable serum levels of the biological.

For etanercept, however, it is unclear whether a relation between clinical response and the formation of antibodies is present in AS patients. In addition, many questions concerning immunogenicity have not been answered yet and different methods of detection of anti-etanercept are being used, which makes the results difficult to compare(5;6).

In our previous studies, we demonstrated a correlation between clinical response and serum trough infliximab levels, adalimumab levels and the onset of antibodies against these drugs(7;8). In this study, we used the same approach as in our previous studies, to investigate the relation between clinical response, functional etanercept levels and the detection of anti-etanercept antibodies in AS patients. Besides, in a few patients the etanercept levels were measured daily to investigate their course over time.

PATIENTS AND METHODS

Patients and study protocol

Consecutive AS patients, attending the outpatient clinics of Jan van Breemen Institute or VU University Medical Center, who were scheduled for treatment with etanercept, were included and followed prospectively. All AS patients fulfilled the modified New York criteria and started using etanercept according to the ASAS consensus statement on the initiation of TNF blocking agents in AS(9). According to this ASAS consensus, patients must have an insufficient response to non-steroid anti-inflammatory drugs (NSAIDs) and a Bath ankylosing spondylitis disease activity index (BASDAI) above 4 (0-10 scale) before starting treatment with etanercept. After tuberculosis was excluded by means of a tuberculin skin test and chest X-rays, subcutaneous injections with etanercept 25mg were taken twice a week. Concomitant medication remained unaltered for at least three months after the start of etanercept treatment. Demographic data collected at baseline were recorded from medical history and patients' medical records. The study was approved by the medical ethical committee and all patients gave their written informed consent.

Outcome measures

Data were collected at baseline, and after 3 and 6 months of treatment. During every visit questionnaires such as BASDAI, patient global disease activity and Bath ankylosing spondylitis functional index (BASFI) were obtained and after three months patients were checked whether they met the ASAS response criteria.

The primary outcome measure was clinical response after 3 months of treatment with etanercept, according to the "International ASAS Consensus Statement for the use of TNF-agents in patients with AS" which is equivalent to the Dutch

guidelines for continuation of TNF blocking agents(1;9-11). In this consensus statement, ASAS response was defined as a 50% improvement or as an absolute improvement of 2 points of the BASDAI (0-10 scale) and an expert opinion in favour of continuation of treatment after 3 months. Routine laboratory tests (ESR, CRP) were performed. A CRP below 8.0 mg/l was considered to be normal.

Etanercept levels and antibodies against etanercept were measured in patients' sera at baseline, and after 3 and 6 months. Furthermore, in five patients serum etanercept levels were measured daily in between two consecutive subcutaneous injections.

Assessment of functional serum etanercept levels

Etanercept levels were measured by means of a newly developed ELISA, based on the principle that etanercept is captured through its ability to bind TNF (Sanquin, Diagnostic Services, Amsterdam, the Netherlands). The sensitivity of detection is 1 ng/ml (=0.001mg/l).

In short, a mouse monoclonal antibody directed against TNF (CLB TNF/5) was coated overnight at room temperature (0.2 µg/ well) on flat bottomed microtitre plates. Recombinant TNF (5 ng/well) in HPE buffer (Sanquin, Diagnostic Services, Amsterdam, The Netherlands) was added and remained in for 1 hour. After washing the plates with phosphate buffered saline/0.02% Tween, patients' serum samples were added in different dilutions in high performance ELISA (HPE) buffer and incubated for 1 hour at 37°C. Plates were washed with phosphate buffered saline/0.04% Tween, and incubated with biotinylated polyclonal rabbit antibodies against etanercept in 100 µl HPE buffer for 1 hour at 37°C. Subsequently, after washing the plates, poly-HRP-conjugated streptavidin was added (30 min at 30°C), followed by incubation with TMB. The reaction was stopped with 2M H₂SO₄. Absorption at 450 nm was

determined, and results were related to a titration curve of etanercept, which was present in each plate. Functionally active etanercept was measured because of its ability to bind TNF.

Assessment of antibodies against etanercept

Two-site assay

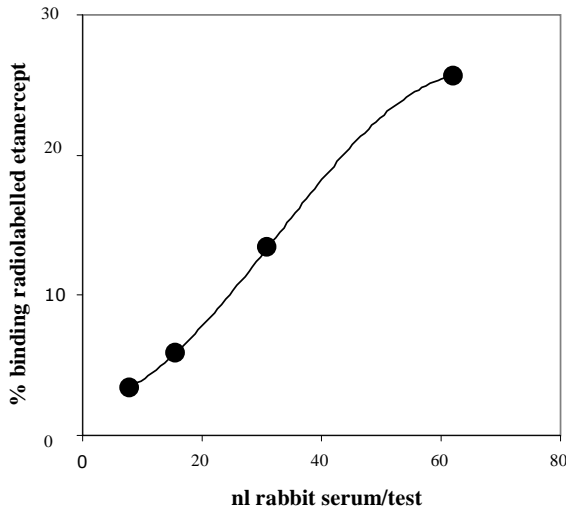
Anti-etanercept antibodies were determined by a two-site assay, in the following manner: etanercept coupled to Sepharose (100 µg/100 ml) was used to bind etanercept-specific antibodies from 1 µl of patient serum, diluted in PBS/BSA (0.3%)/Tween (0.2%), during an overnight incubation on a rotator. After removal of non-bound serum components by washing, ¹²⁵I-radiolabelled etanercept (~ 1 ng per test) was added, followed by a second overnight rotating incubation in Freeze buffer (Sanquin, Amsterdam, the Netherlands). The non-bound radiolabel was washed off, and test results were compared to a calibration curve made of serum from an etanercept-vaccinated rabbit (Figure 1). Cutoff level for a positive signal was set at 0.95% binding (mean+3SD of the pre-treatment values).

Antigen binding test

Besides, testing for antibodies was carried out by using an antigen binding test, which is in principle similar to the tests we routinely use for detection of antibodies against infliximab(12;13).

Pepsin-treated ¹²⁵I-radiolabelled etanercept was used for the detection of antibodies. The same rabbit-derived calibration curve as applied in the two-site assay was used for the interpretation of patients' results. Cutoff level for a positive signal was set at 1.07% binding (mean+3SD of the pre-treatment values).

Figure 1: Calibration curve of rabbit anti-etanercept serum as a positive control for the two-site assay.



Statistical analysis

The last observation was carried forward in patients who had dropped out before 6 months of treatment, due to ASAS non-response, adverse events or loss to follow-up.

Data were expressed as mean (SD) or median (interquartile range) where appropriate. The distribution of variables was tested for normality and transformed if possible. Independent t-tests were used for variables with a normal distribution and nonparametric tests (Wilcoxon signed-rank test or Mann-Whitney U test) for skewed variables. Pearson χ^2 tests were conducted for dichotomous variables.

Logistic regression analyses were conducted to examine associations between ASAS response and serum etanercept level, and were corrected for the possible influence of demographic, clinical and laboratory variables. Subsequently, a linear regression model was used to investigate whether serum etanercept levels

were associated with any of the demographic, clinical or laboratory variables. The following variables were tested: sex, age, ethnicity, BMI and HLA-B27, baseline bodyweight, baseline BMI, decrease of Patient Global assessment, absolute decrease of ESR and CRP level. Calculations were made using SPSS 14.0 software. The threshold for significance was set at $p < 0.05$.

RESULTS

Fifty-three patients were included in this study in the period of July 2004 until March 2006. The demographic data and baseline characteristics are shown in Table 1.

Table 1: Demographic data and baseline characteristics.

<i>Variables</i>	<i>Baseline</i>	<i>3 Months</i>	<i>6 Months</i>
Male sex, N (%)	40 (76)		
Age (years)	41 (11)		
Caucasian, N (%)	45 (85)		
BMI	25 (4.4)		
HLA-B27 positivity, N (%)	44 (88)		
Peripheral arthritis, N (%)	28 (53)		
BASDAI	6.4 (1.3)	3.1 (2.0) *	2.5 (1.7) *
Patient global disease activity VAS	7.2 (1.9)	3.2 (2.4) *	2.5 (2.1) *
BASFI	6.2 (2.1)	4.1 (2.5) *	3.5 (2.5) *
ESR (median, range)	22 (1-114)	5 (1-58) *	4 (1-33) *
CRP (median, range)	17 (1-92)	4 (1-44) *	4 (1-74) *
Serum etanercept levels	0	2.7 (1.2)	3.0 (1.0)
Antibodies to etanercept	0	0	0

Except when indicated otherwise, the values are the mean (SD). * Significance level: $p < 0.001$

BMI, body mass index; HLA-B27, human leukocyte antigen B27; BASDAI, Bath ankylosing spondylitis disease activity index (0-10); BASFI, Bath ankylosing spondylitis functional index (0-10); VAS, visual analogue scale; ESR, erythrocyte sedimentation rate, normal < 10 mm/hour; CRP, C-reactive protein, normal < 8.0 mg/l; mean serum etanercept levels (mg/l); antibodies to etanercept (mg/l).

Bias due to missing data was excluded, for ASAS response did not differ between patients with available data and those with missing data at 6 months. Treatment with etanercept resulted in significant clinical improvement after 3 and 6 months, when compared to baseline (Table 1). The mean BASDAI, Patient global disease activity, BASFI and ESR were even lower after 6 months of treatment. Baseline characteristics did not differ significantly for responders and non-responders (Table 2).

After 3 months of treatment, 40 of the patients (76%) met the ASAS response criteria. The etanercept levels were measured in 48 patients after 3 months of treatment and in 41 patients after 6 months of treatment: ten of 41 samples turned out to be samples of ASAS non-responders. These patients should have stopped treatment with etanercept after 3 months because of insufficient improvement of the BASDAI. However, they continued their treatment based on the expert opinion of the rheumatologist.

At baseline serum etanercept levels were undetectable, mean serum etanercept levels were 2.7 mg/l (SD 1.2 mg/l) and 3.0 mg/l (SD 1.0 mg/l) after 3 and 6 months of treatment respectively. In each patient etanercept levels could be measured and were within the same range (Figure 2). The mean etanercept levels of responders and non-responders were similar, which could be explained by the fact that antibodies against etanercept were not detected in these patients with any of the assays (Table 2 and Figure 2). Besides the lack of association between clinical response and serum etanercept levels, there also turned out to be no significant association between sex, age, ethnicity, HLA-B27, baseline bodyweight, baseline BMI, decrease of Patient Global assessment, absolute decrease of ESR and CRP level, and serum etanercept levels.

In addition, in five patients etanercept levels were measured at daily intervals between two subsequent injections with a mean of 3.5 mg/l, SD 1.2 mg/l and a variance of 1.5. Furthermore, sera of six non-responders were taken three

months after the final etanercept injection and, as expected, etanercept was no longer present in these sera and antibodies against etanercept could not even be detected in these patients.

Table 2: Baseline characteristics, baseline ESR and CRP, and etanercept levels after 3 months of treatment for ASAS responders and non-responders.

<i>Variables</i>	<i>Responders N = 40</i>	<i>Non-responders N = 13</i>	<i>Significance level</i>
Male sex, N (%)	29 (73)	11 (85)	0.38
Age in years	41 (11.2)	41 (8.8)	0.82
Caucasian, N (%)	36 (90)	9 (69)	0.07
BMI	25 (4.8)	24 (3.1)	0.53
HLA-B27 positivity, N (%)	34 (90)	10 (83)	0.57
Peripheral arthritis, N (%)	21 (53)	7 (54)	0.99
BASDAI	6.4 (1.3)	6.2 (1.4)	0.57
Patient global disease activity VAS	7.1 (1.5)	7.4 (1.9)	0.31
BASFI	6.2 (2.1)	6.2 (2.2)	0.86
ESR (median, range, mm/h)	22 (1-114)	22 (1-87)	0.82
CRP (median, range)	18 (1-91)	10 (1-92)	0.45
Etanercept level (mg/l)	2.7 (1.3)	2.8 (1.1)	0.50

Except when indicated otherwise, the values are the mean (SD).

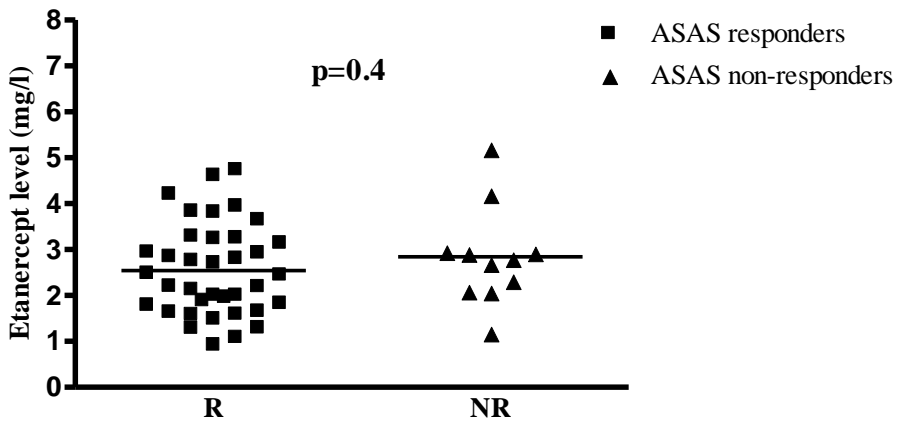
BMI, body mass index; HLA-B27, human leucocyte antigen B27; BASDAI, Bath ankylosing spondylitis disease activity index; VAS, visual analogue scale; BASFI, Bath ankylosing spondylitis functional index; ESR, erythrocyte sedimentation rate, normal<10 mm/hour; CRP, C-reactive protein, normal<8.0 mg/l.

Adverse events

One patient had an adverse reaction of flushing and dyspnea after 2 months of treatment with etanercept. Another patient developed urticaria and atopic dermatitis after 27 months of treatment with the prefilled syringe of etanercept, however, this patient showed none of these symptoms after retreatment with

etanercept in the form of lyophilized powder. Both patients had previously developed an infusion reaction to infliximab after which they had switched to etanercept. No antibodies against etanercept were detected in any of the patients with an adverse reaction.

Figure 2: Etanercept levels (mg/l) for ASAS responders and non-responders after 3 months of treatment.



DISCUSSION

Seventy-six percent of the AS patients were classified as responders to etanercept after 3 months of treatment, which is comparable to the response rate observed in clinical trials. No correlation was found between etanercept levels, formation of antibodies against etanercept and clinical response. All patients had detectable serum levels of etanercept and no antibodies against etanercept were found. Interestingly, there seemed to be no difference in mean etanercept levels and the onset of antibodies against etanercept between responders and non-responders. These findings are in contrast with our previous studies with infliximab(8;13) and adalimumab(7) in RA and AS, where a strong relation was found between a decreased response and the onset of antibodies against TNF

blocking agents. These antibodies against TNF blocking agents often resulted in low or absent serum levels of these drugs which would explain the decrease in efficacy, but apparently this is not the case in etanercept. Therefore, this study seems to confirm the hypothesis that etanercept is less immunogenic than other TNF blocking agents.

Although etanercept levels have not yet been correlated with clinical response, some studies do report on the detection of antibodies against etanercept. In these, etanercept has been labelled as having antibody formation in less than 6% of the cases and no clear relation with clinical response was detected(5;6;14). These discrepancies regarding anti-therapeutic drug antibodies could be explained by the use of different detection methods. ELISA is known to give more false positive signals than antigen binding assays and it is therefore difficult to compare other results with our method. The detection method for anti-etanercept used by our laboratory may be regarded as not very sensitive. However, this does not agree with the fact that functional etanercept levels could be measured in all patients, because the presence of antibodies would have resulted in an enhanced clearance and removal of etanercept. Furthermore, similar techniques for measuring functional etanercept levels and detecting of antibodies against etanercept were proven to be sensitive in our previous studies with other TNF blocking agents.

Several arguments are in favour of the hypothesis that etanercept shows less immunogenicity compared with other TNF-inhibitors. Firstly, etanercept has a less immunogenic structure compared with the other TNF blocking agents. Etanercept is a dimeric fusion protein consisting of two TNF receptors, linked to the Fc portion of an immunoglobulin (Ig)G1. Only the fusion part of the molecule may contain immunogenic epitopes. Infliximab is a chimerical monoclonal IgG1 antibody against TNF, partly consisting of murine protein. Adalimumab is a fully human monoclonal antibody against TNF. These

monoclonal antibodies have more epitopes within the variable region of the antibody to which an immune response can be directed. Secondly, major fluctuations in serum levels may precipitate an immune response and the development of antibodies against the TNF blocking agent(15). This is mainly the case in treatment with infliximab, which is administered once every six to eight weeks. Treatment with etanercept, however, produces stable levels between two injections and is dosed much more frequently, which is in line with previous findings(16-19). The variance of 1.5 of the etanercept levels measured at daily intervals between two subsequent injections measured in our five patients was very low, compared with the variance of all serum trough infliximab levels measured in our previous study, which was 80. The overall variance of all etanercept levels was 1.3, which is also very low. Thirdly, there may be different mechanisms for non-response. Theoretically, non-responders can be divided into two categories: true non-responders, whose illness is not mainly caused by excess production of TNF, and non-responders whose illness is caused by inadequate blocking of TNF. The latter can be caused by enhanced clearance or as a result of inadequate dosing. A dose-response relation of etanercept in AS has not been investigated before. There is a possibility that small differences in etanercept levels could not be found because of the random timing of sampling between two injections. The most important argument against this view is probably the observation that etanercept levels, measured at several time intervals between two subsequent injections in the five patients, were quite stable. Furthermore, other authors have confirmed that etanercept levels are likely to be stable between two injections(18). Additionally, the mean etanercept levels were equal in both responders and non-responders and this measurement error would influence both groups in the same way.

To conclude, all AS patients had detectable etanercept levels, regardless of whether they were responders or non-responders. In contrast with previous studies with other TNF blocking agents, no antibodies against etanercept were detected with any of the assays. To summarise, this study indicates that immunogenicity does not play an important role in explaining the non-response of AS patients to treatment with etanercept.

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Section II

**Biomarkers of disease activity
and cardiovascular risk**

5.1

Erythrocyte sedimentation rate, C-reactive protein level, and serum amyloid A protein for patient selection and monitoring of anti-tumor necrosis factor treatment in ankylosing spondylitis.

Mirjam K. de Vries¹, Izhar C. van Eijk³, Irene E. van der Horst-Bruinsma¹, Mike J. L. Peters^{1,3}, Michael T. Nurmohamed^{1,3}, Ben A.C. Dijkmans^{1,3}, Bouke P.C. Hazenberg⁴, Gerrit Jan Wolbink^{2,3}.

Rheumatology Department VU University Medical Center¹, Immunopathology
Department Landsteiner Laboratory Sanquin Research², Rheumatology
Department Jan van Breemen Institute³, Amsterdam, Rheumatology
Department University Medical Center Groningen⁴, the Netherlands.

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ABSTRACT

Objectives: To study the usefulness of erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), and serum amyloid A (SAA) for response prediction and monitoring of anti-tumor necrosis factor (anti-TNF) treatment in ankylosing spondylitis (AS) patients.

Methods: Patients were included consecutively before starting etanercept or infliximab treatment. Assessment in ankylosing spondylitis (ASAS) response, defined as a 50% improvement or an absolute improvement of 2 points of the Bath ankylosing spondylitis disease activity index (BASDAI; 0-10 scale), was assessed at 3 months. Inflammatory markers and the BASDAI were collected at baseline and 1 and 3 months. Longitudinal data analysis was performed to compare associations between inflammatory markers and the BASDAI over time by calculating standardized betas. Predictive values of baseline levels of inflammatory markers for ASAS response were calculated.

Results: In total, 155 patients were included, of whom, after 3 months of treatment, 70% in the etanercept cohort and 71% in the infliximab cohort responded. All markers, notably SAA, decreased significantly ($p < 0.0001$). Standardized betas were 0.49 for ESR, 0.43 for CRP and 0.39 for SAA. Normal baseline levels of CRP and SAA were significantly associated with non-response. A combination of elevated CRP and SAA levels at baseline revealed the highest predictive value (81%) for ASAS response.

Conclusion: ESR, CRP and SAA were significantly associated with the BASDAI over 3 months, and the association with ESR was the strongest. Elevated baseline CRP and SAA levels revealed the highest predictive value for response. Together, this study demonstrates that inflammatory markers, and notably CRP and SAA, may facilitate patient selection and monitoring of efficacy of anti-TNF treatment in AS, and could be added to response criteria.

INTRODUCTION

Disease activity in ankylosing spondylitis (AS) is generally measured with the Bath ankylosing spondylitis disease activity index (BASDAI)(1). Despite the fact that the BASDAI is a validated instrument used in many clinical trials as an outcome parameter for disease activity, it remains a subjective parameter that is based on a patient questionnaire. A previous study showed that the BASDAI has a high intra-individual week-to-week variability(2). Theoretically, a high BASDAI can be caused by 3 factors, including a high level of a) ankylosis or joint destruction, b) psychological stress, or c) inflammation. There is an unmet need for more objective biomarkers of disease activity in AS similar to the erythrocyte sedimentation rate (ESR) or C-reactive protein (CRP) level in rheumatoid arthritis (RA). ESR and CRP level are sensitive markers of disease activity in RA and are a reflection of the plasma levels of pro-inflammatory cytokines, rendering them suitable for monitoring the effectiveness of anti-tumor necrosis factor (anti-TNF) drugs. In AS, however, the sensitivity of these inflammatory markers as biomarkers of disease activity is controversial(3).

On one hand, ESR and CRP level are poorly associated with disease activity in AS, and on the other hand, they may help a clinician to predict the response on TNF blockers(4-7). Two studies reported a strong association between ESR or CRP levels at baseline and clinical response to treatment with anti-TNF after 3 months, supporting a potential distinctive exploitation of these biomarkers in identifying AS patients suitable for treatment with anti-TNF(8;9), which is also of particular relevance in light of the costs of biologics and the side effects of these drugs. However, because changes of CRP level may be too small to be detected in AS with common methods, measurement of high-sensitivity CRP (hsCRP) might be a more appropriate marker for disease activity. Besides ESR and CRP, other inflammatory markers are known, such as serum amyloid A

protein (SAA)(10). SAA is an acute-phase reactant that is mainly transported as an apolipoprotein in high-density lipoprotein and is predominantly synthesized in the liver by hepatocytes in response to proinflammatory cytokines(11). SAA was shown to correlate with disease activity in AS, and one study even suggested superiority of SAA to ESR and CRP(4;12). However, longitudinal data and the effects of treatment with anti-TNF agents on these relation are lacking. Therefore, we explored the usefulness of ESR, CRP, hsCRP, and SAA for monitoring inflammation in AS patients treated with anti-TNF along with the association between these inflammatory markers and the BASDAI over time. In addition, the relation between elevated levels of these markers at baseline and assessment of ankylosing spondylitis (ASAS) response was studied in these patients after 3 months of treatment with etanercept or infliximab in order to predict efficacy of this treatment.

PATIENTS AND METHODS

Patients and study protocol

Consecutive AS patients attending the outpatient clinics of Jan van Breemen Institute and VU University Medical Center (VUmc) scheduled for treatment with etanercept were included and followed prospectively, as well as AS patients scheduled for treatment with infliximab at VUmc.

All patients fulfilled the 1984 modified New York criteria(13) and started anti-TNF therapy according to the ASAS consensus statement on the initiation of TNF blocking agents in AS(14). Patients were treated with 25 mg etanercept twice a week, 50 mg etanercept once a week, or infliximab 5 mg per kilogram of body weight every 6 weeks after a starting regimen. None of the patients was treated with adalimumab because adalimumab was not reimbursed for yet at the start of this study.

The study was approved by the medical ethical committees of both participating centers, and all of the patients gave written informed consent.

Outcome measures

The primary outcome measure was clinical response after 3 months of treatment with etanercept or infliximab, according to the international ASAS consensus statement for the use of TNF agents in patients with AS, which is equivalent to the Dutch guidelines for the continuation of TNF blocking agents. In this consensus statement, the ASAS response was defined as a 50% improvement or as an absolute improvement of 2 points of BASDAI (0-10 scale), and an expert opinion in favour of continuation of treatment after 3 months(15;16).

Data and sera were collected at baseline and after 1 and 3 months of treatment. During every visit, questionnaires on disease activity (BASDAI) were obtained. ESR and CRP level were routinely determined. HsCRP and SAA were measured in a patient's sera at baseline and 1 and 3 months. Collected sera were frozen at -20°C until testing. Commercially available kits were used to measure these inflammatory markers.

Analysis of ESR, CRP, hsCRP, and SAA

ESR was measured with the Westergren method. Values are expressed in mm/hour. An ESR <15 mm/hour was considered to be normal, according to the cutoff used at the Jan van Breemen Institute.

Serum CRP levels were determined using the Roche/Hitachi Modular P (VU University Medical Center) or cobas c (Jan van Breemen Institute) analyzers (Roche Diagnostics, D-68298, Mannheim, Germany), based on the principle of particle-enhanced immunologic agglutination. Values are expressed in mg/liter. A CRP level <10 mg/liter was considered to be normal, according to the cutoff used at the Jan van Breemen Institute.

HsCRP levels were determined using the Roche/Hitachi cobas c systems (Roche Diagnostics), with a detection range of 0.15-20 mg/liter. The test principle consists of a particle-enhanced immunoturbidimetric assay. Human CRP agglutinates with latex particles coated with monoclonal anti-CRP antibodies. SAA levels were assessed with an enzyme-linked immunosorbent assay, as previously described(17). A value <4 mg/liter was considered to be normal.

Statistical analysis

Continuous variables were reported as the mean \pm SD or, if skewed, as the median (interquartile range). Categorical variables were calculated as frequencies and percentages. The distribution of variables was tested for normality and transformed if necessary and possible. Because the distribution of all the inflammatory markers was skewed, Wilcoxon's signed rank test was performed to investigate paired samples. Relative changes (%) of inflammatory markers were calculated. Generalized estimating equations (GEEs) were performed to investigate the longitudinal relationship between inflammatory markers and BASDAI over a period of 3 months by calculating standardized betas. We investigated the possible influence of demographic or clinical variables, i.e., sex, age, ethnicity, HLA-B27, presence of peripheral arthritis, and disease duration.

Logistic regression analysis was performed to investigate the association between the baseline levels of the inflammatory markers and the dichotomous outcome variable of ASAS response. Odds ratios (ORs) and 95% confidence intervals (95% CIs) were calculated for the association between elevated baseline levels of the inflammatory markers and ASAS response. For ESR, CRP and SAA as predictors of ASAS response, the sensitivity and specificity were calculated and receiver operating characteristic (ROC) curves were constructed.

In addition, predictive values of normal or elevated levels of CRP and/or SAA were calculated for ASAS response. Statistical analyses were performed with SPSS statistical software, version 15.0 (SPSS, Chicago, IL). The threshold for significance was set at p values less than 0.05.

RESULTS

In total, 155 patients were included and monitored after starting anti-TNF treatment. The demographic and clinical features are shown in Table 1. During treatment, all pharmacologic treatment remained unchanged.

Table 1: Demographic and clinical assessments of ankylosing spondylitis patients at baseline and 1 and 3 months of treatment with etanercept or infliximab.

<i>Variables</i>	<i>Baseline N=155</i>	<i>1 Month</i>	<i>3 Months</i>
Male sex, N (%)	101 (65)		
Age, mean \pm SD years	42 \pm 11		
White, N (%)	125 (81)		
HLA-B27 positive, N (%)	123 (85)		
Presence of peripheral arthritis, N (%)	88 (58)		
Disease duration, years	8 (3-16)		
BASDAI (0-10 scale)	6.2 (5-7.1)	3.4 (1.8-5.7) †	2.8 (1.4-4.3) †
ESR, mm/hour (normal value <10)	21 (7-38)	6 (2-12) †	5 (2-13) †
CRP level, mg/liter (normal value <10)	15 (5-38)	3 (1-5) †	4 (2-6) †
HsCRP level, mg/liter (normal value <10)	14.3 (3.8-39.2)	1.9 (0.8-4.8) †	1.9 (0.9-5.8) †
SAA level, mg/liter (normal value <4)	7.5 (1.9-26.0)	0.7 (0.2-1.8) †	0.8 (0.2-2.0) †

Values are the median (interquartile range) unless otherwise indicated. BASDAI; Bath ankylosing spondylitis disease activity index; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; hsCRP, high-sensitivity CRP; SAA, serum amyloid A protein. †p<0.0001 compared with baseline.

The etanercept cohort comprised 117 patients and the infliximab cohort comprised 38 patients. After 3 months, 70% (80 of 115, because in 2 patients the ASAS response was missing) and 71% (27 of 38) of patients achieved ASAS response in the etanercept and infliximab cohort, respectively. Baseline levels of ESR, CRP, and SAA were elevated in 113 (73%), 96 (62%), and 99 (64%) patients, respectively. In 29 patients (19%), none of these markers was elevated at baseline. All of the inflammatory markers and the BASDAI decreased significantly after starting anti-TNF therapy ($p<0.0001$) (Table 1 and Figure 1). Notably, the median relative decrease after 1 month was 36% for the BASDAI (Figure 1A), 67% for the ESR (Figure 1B), 75% for the CRP (Figure 1C), and 84% for the hsCRP (Figure 1D), while SAA decreased by 90% (Figure 1E). Longitudinal linear regression analysis (GEE) showed significant association between BASDAI and ESR, CRP, hsCRP and SAA over time ($p<0.0001$). There were no confounders influencing this association. The standardized betas were 0.49, 0.43, 0.43, and 0.39 for ESR, CRP, hsCRP and SAA, respectively. Hereafter, only results of associations with CRP level are shown as results because the hsCRP level did not differ from the CRP level.

In 2 patients treated with infliximab who showed a secondary increase, particularly CRP and SAA after initial normalization, antibodies against infliximab were detected (Figure 2)(18).

Patients with an elevated baseline level of CRP (>10 mg/liter) achieved an ASAS response after 3 months of treatment significantly more often compared with patients with normal baseline CRP levels (OR 2.8, 95% CI 1.3-5.7, adjusted for sex and age). Elevated baseline SAA levels had a similar association with ASAS response at 3 months of treatment with either infliximab or etanercept (OR 2.9, 95% CI 1.4-6.1, adjusted for sex and age). Only baseline ESR levels were not significantly associated with clinical response (OR 1.4, 95% CI: 0.7-3.1).

Figure 1: Change of BASDAI (A), ESR (B), CRP (C), hsCRP (D) and SAA (E) after 1 and after 3 months of treatment with etanercept or infliximab.

IQR, interquartile range; * $p < 0.0001$ compared to baseline.

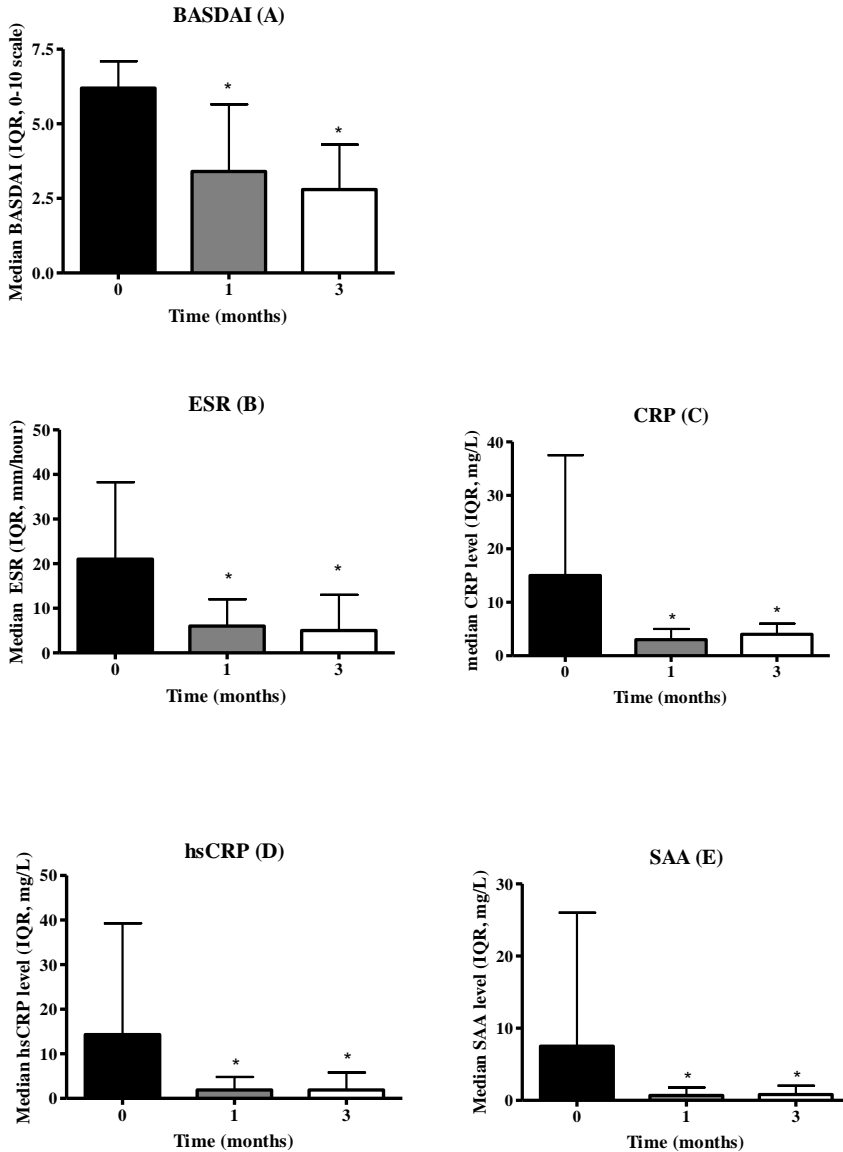
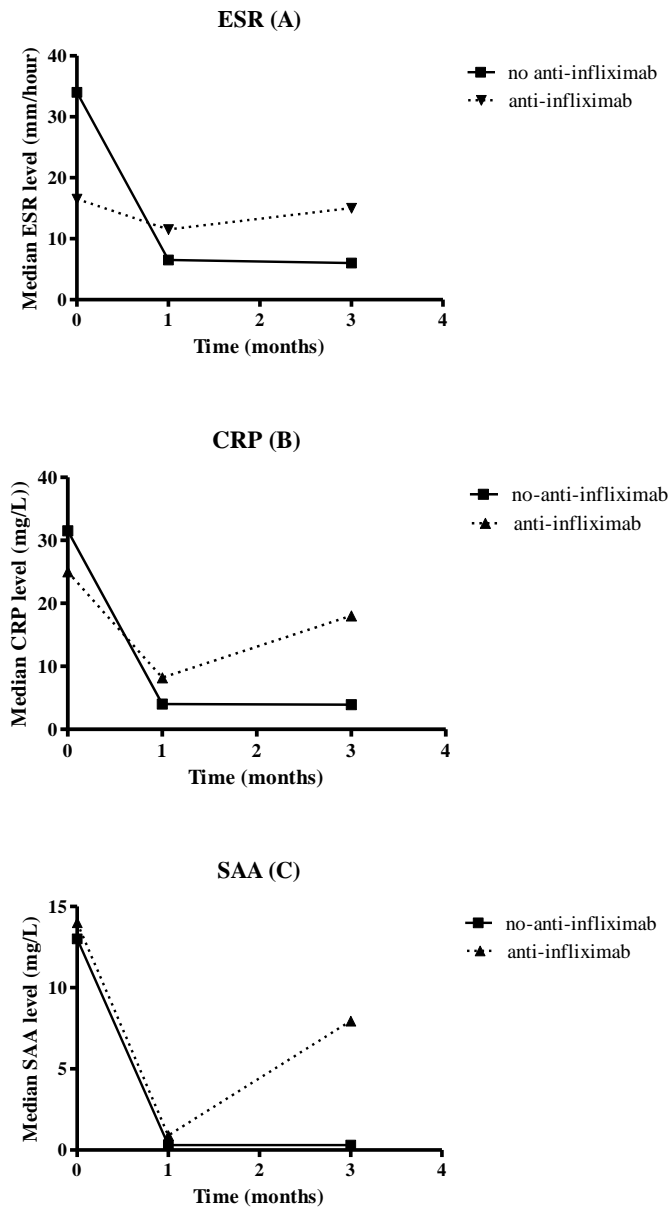


Figure 2: ESR (A), CRP (B) and SAA (C) levels 1 and 3 months after initiation of treatment with infliximab for AS patients without and with antibodies against infliximab (anti-infliximab, N=2).



The sensitivity and specificity of CRP for prediction of ASAS response were 0.69 and 0.57, respectively. These figures were 0.72 and 0.54 for SAA level, respectively. ROC curves were created for ESR, CRP, and SAA as predictors of ASAS response (Figure 3). The ROC curve for ESR (Figure 3A) showed that determination of ESR is of no additional value in predicting ASAS response. CRP and SAA performed similarly (Figures 3B and 3C).

Figure 3: Receiver operating characteristic (ROC) curves for A, ESR (area under the curve [AUC] 0.55), B, CRP (AUC 0.64), and C, SAA (AUC 0.66) as predictors of the assesment in ankylosing spondylitis response.

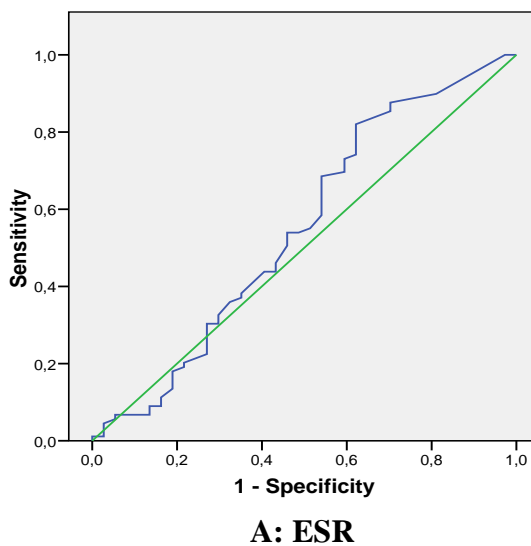


Figure 3B: ROC CRP (AUC 0.64)

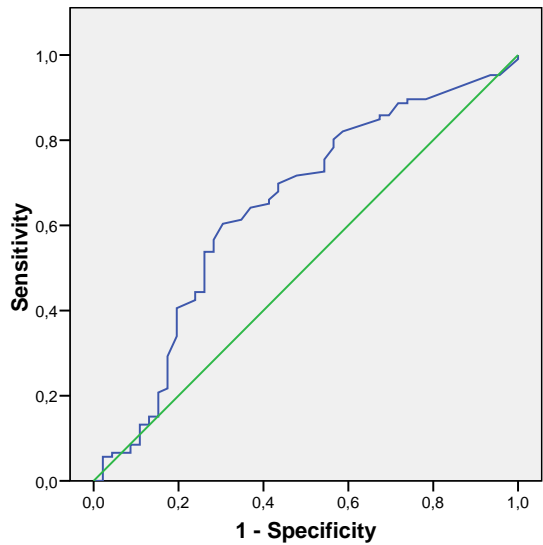
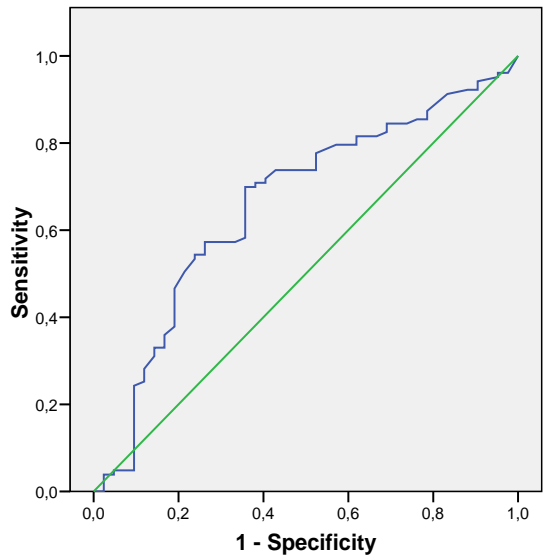


Figure 3C: ROC SAA (AUC 0.66)



The predictive value of ASAS response of an elevated baseline level of CRP was 79%. This value was identical to SAA. A combination of the presence of elevated baseline levels of CRP and SAA of patients in both cohorts displayed the highest predictive value of ASAS response of 81% (Table 2). Inclusion of baseline levels of ESR had no additional value. A proportion of 48% of patients with normal values of both CRP and SAA levels at baseline were non-responders according to the ASAS criteria.

Table 2: Predictive values of normal or elevated pretreatment levels of CRP and/or SAA for prediction of ASAS response/non-response after 3 months of treatment with etanercept or infliximab.

	<i>ASAS non-response, %</i>	<i>ASAS response, %</i>
Normal CRP (N=59)	44	56
Elevated CRP (N=94)	21	79
Normal SAA (N=55)	45.5	54.5
Elevated SAA (N=98)	21	79
Normal CRP and SAA (N=44)	48	52
Elevated CRP and normal SAA (N=11)	36	64
Normal CRP and elevated SAA (N=15)	33	67
Elevated CRP and SAA (N=83)	19	81

CRP, C-reactive protein; SAA, serum amyloid A protein; ASAS, assessment in ankylosing spondylitis.

DISCUSSION

The present study demonstrated that a combination of elevated baseline levels of CRP and SAA can be a valuable instrument for the selection of those AS patients who are likely to respond to treatment with anti-TNF, unlike the BASDAI. Moreover, inflammatory markers, CRP and SAA in particular, seem useful for monitoring the level of inflammation in patients with AS who are treated with etanercept or infliximab.

Regarding monitoring therapy with anti-TNF, most of the AS patients showed a significant decrease of several inflammatory markers, most dominantly SAA. In some cases, a secondary increase of these inflammatory markers can be seen, which might be caused by a concurrent infection or inadequate therapeutic levels. The latter may be due to antibody formation, which depletes anti-TNF levels below therapeutic thresholds(18). This could be an argument to include at least one inflammatory marker next to the BASDAI in order to assess disease activity properly. Although ESR showed the strongest association with BASDAI over time, we consider ESR the least suitable for inclusion because it has no additional value to the BASDAI, as the half-life of this inflammatory marker is too long for early detection of changes. HsCRP has been proposed to be useful as a marker to predict the risk of coronary heart disease due to inflammation in apparently healthy persons(19). In the current study, however, measurement of hsCRP level did not provide additional value because the strength of the association with disease activity of AS patients over time was not different from and not superior to that of CRP.

Although the majority of the AS patients in this study (62% to 73%) had elevated inflammatory markers before the start of anti-TNF therapy, it is known that inflammatory markers do not necessarily reflect disease activity well in AS(3). This is why inflammatory markers were not implemented for assessment

of disease activity or response to treatment, which is in contrast to RA. This study shows that when inflammatory markers are raised, this is indicative of active disease. It seems useful to add the decrease of inflammatory markers to response criteria for continuation of anti-TNF treatment in AS patients who show elevated inflammatory markers at baseline.

Since anti-TNF therapy is not without risks and is also very costly, it is of great importance to identify patients likely to (non-)respond to this type of drug. Although the performance of the tests was poor, the ROC curves showed that CRP and SAA are superior to ESR. In the present study, we showed that the combination of elevated CRP and SAA levels at baseline is the strongest predictor of ASAS response, providing a solid basis for a predictive assessment of the clinical response of AS patients to treatment with anti-TNF. In contrast, baseline ESR levels were not associated with clinical response. However, as demonstrated before, patients with normal baseline levels of CRP and SAA may respond to anti-TNF therapy as well(20). Therefore, at this moment, we believe inflammatory markers can be very useful as one of the predictors of a good response, but a raise of the inflammatory markers should not be mandatory for allowing AS patients to be treated with anti-TNF.

The fact that good responders do not all necessarily need to have a strong decline of CRP or SAA levels limits the use of these parameters in making the decision of whether or not this therapy should continued. Therefore, they can be useful, but should not be considered obligatory for the decision of whether anti-TNF therapy is failing or not.

We studied SAA in relation to disease activity in AS. SAA is implicated in several chronic inflammatory diseases, such as AA amyloidosis, atherosclerosis, and RA(21). An additional advantage of monitoring SAA levels in AS patients may therefore be that SAA lowering therapy with anti-TNF could possibly prevent secondary AA amyloidosis. AA amyloidosis sometimes develops

secondary to longstanding inflammation and chronically elevated levels of SAA, the plasma precursor of amyloid A deposits(10). Notably, elevated baseline levels of SAA were associated with clinical response in AS and decreased rapidly after initiation of treatment with etanercept or infliximab, which might prevent AA amyloidosis in the future.

Altogether, in this large prospective cohort of AS patients, measurement of inflammatory markers, in particular CRP and SAA, served as a powerful tool not only for monitoring the efficacy of anti-TNF therapy, but also for the selection of AS patients with a high likelihood of responding to anti-TNF treatment.

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5.2

C-reactive protein polymorphisms and haplotypes influence serum C-reactive protein levels independent of disease activity in ankylosing spondylitis.

Theodora A.M. Claushuis¹, Mirjam K. de Vries^{*1}, Mignon A.C. van der Weijden^{*1}, Ingrid M. Visman², Michael T. Nurmohamed², Jos W.R Twisk³, Irene E. Van der Horst-Bruinsma¹, Jakob B.A. Crusius⁴.

^{*} both authors contributed equally to this paper

Rheumatology Department VU University Medical Center¹, Rheumatology Department Jan van Breemen Research Institute/Reade², Methodology and Applied Biostatistics Department VU University³, Laboratory for Immunogenetics at the Medical Microbiology and Infection Control Department VU University Medical Center⁴, Amsterdam, The Netherlands.

Submitted

ABSTRACT

Objectives: C-reactive protein (CRP) levels are frequently used for determination of disease activity in patients with ankylosing spondylitis (AS), but these levels do not necessarily reflect disease activity. We investigated whether common single-nucleotide polymorphisms (SNPs) and haplotypes in the *CRP* gene influence CRP levels in AS patients. Additionally, the relation between CRP levels and Bath ankylosing spondylitis disease activity index (BASDAI) was examined.

Methods: This exploratory cross-sectional study included 189 Dutch Caucasian AS patients. *CRP* SNPs rs2794521, rs3091244, rs1800947 and rs876538 were genotyped and haplotypes constructed. CRP levels were determined by standard assay. Multivariate linear and logistic regression analyses were used to investigate the relation between SNPs and CRP levels, adjusting for NSAID use, BMI, smoking status, age, sex and BASDAI to correct for disease activity.

Results: CRP levels were significantly positively correlated with BASDAI ($p=0.001$). AS patients with genotype CA of the tri-allelic (C>T>A) SNP rs3091244 were associated with significantly higher CRP levels when compared with genotype CC (CA: 18.6 mg/l vs. CC: 8.3 mg/l; $p=0.02$). Carriers of haplotype 5 (tagged by allele A of rs3091244) had a significantly higher odds ratio when compared to non-carriers to express a $CRP \geq 10$ mg/l (OR=2.9, 95%CI 1.0-8.3; $p=0.05$) in the multivariate regression analyses.

Conclusions: In AS, genotypes and the haplotype tagged by allele A of rs3091244 associate with high CRP levels, independent of BASDAI and other confounders. Therefore, carrying distinct genetic variants might explain the lack of elevated CRP levels despite high disease activity in certain AS patients and could be important to interpret disease activity scores that incorporate CRP levels, like the ankylosing spondylitis disease activity score (ASDAS).

INTRODUCTION

Ankylosing spondylitis (AS) is a chronic inflammatory rheumatic disease, characterized by sacroiliitis with low back pain and stiffness, restricted motion of the spine and chest expansion due to inflammation and bone formation. Unlike other rheumatic diseases, such as rheumatoid arthritis, it is difficult to assess clinical disease activity in AS, as patients may not exhibit any peripheral arthritis (rendering a 44 joint count unsuitable) and absence of increased inflammatory parameters (such as C-reactive protein (CRP)).

CRP is a plasma protein of the pentraxin family, known for its rapid increased expression as part of the acute phase reaction and CRP levels are widely used in the evaluation of infection or systemic inflammation. The CRP level is only elevated ($\geq 10\text{mg/l}$) in approximately 50% of AS patients with active disease(1-3). Since 1994, disease activity in AS has been measured with the Bath ankylosing spondylitis disease activity index (BASDAI), a patient orientated questionnaire(4). Active disease is specified as a BASDAI ≥ 4 (on a 0-10 scale) and is used as an indicator to start treatment with TNF blocking agents.

Recently, the assessment of ankylosing spondylitis (ASAS) reported the ankylosing spondylitis disease activity score (ASDAS), a score that incorporates plasma levels of CRP(5). This new scoring system necessitates to obtain information about the value of CRP in AS. CRP levels correlate with the BASDAI but several studies found that these levels do not necessary reflect disease activity in all patients(2;6;7). As far back as 1999 Ruof and Stucki stated that many patients have normal or only minimally increased levels of acute phase reactants(6). A reason for the lack of increased CRP levels in some patients with high disease activity might be a genetic contribution to CRP levels.

In a healthy population, small variations in the low range of plasma CRP (<10 mg/l) were attributed to genetic factors as well as environmental factors such as body mass index (BMI), sex, age and smoking(8). Twin and family studies have illustrated that genetic factors can contribute up to 40-50% of the phenotypic variation of baseline plasma CRP concentrations(9;10). A meta-analysis of genome wide association studies (GWAS) in more than 80.000 individuals identified associations of CRP levels with single-nucleotide polymorphisms (SNPs) at seven loci within or close to the *CRP* gene and six other genes as previously reported in a GWAS scan by Ridker et al.(11) and with 11 novel loci(12). A weighted genetic risk score explained about 5% of the variation in CRP levels.

To date, most disease association studies investigated the influence of *CRP* gene polymorphisms on CRP levels in relation to cardiovascular disease(13-18).

In these studies SNPs and their haplotypes in the *CRP* gene have been identified that modify CRP levels. However, the causal relationship between *CRP*-genetics and cardiovascular disease risk remains controversial(8).

When compared with cardiovascular disease, higher ranges of serum CRP levels are used in the clinical evaluation of AS patients. However, since CRP levels do not always correlate with disease activity in every AS patient we investigated the contribution of *CRP* gene polymorphisms and haplotypes to these CRP levels in AS patients. This influence is of clinical importance as it can interfere with diagnostic and treatment choices based on CRP levels.

Additionally, the association between CRP levels and the BASDAI as a measure of disease activity was studied.

PATIENTS AND METHODS

Patients

A cross-sectional study in AS patients was conducted. The study population included patients treated with tumor necrosis factor (TNF) blocking agents (infliximab, etanercept or adalimumab) and a group of anti-TNF-naïve patients. All AS patients fulfilled the 1984 modified New York criteria(19), were self-reported Dutch Caucasians and unrelated to any other patient participating in this study. Patients were recruited from the VU University Medical Center (VUmc) and Reade, formerly known as the Jan van Breemen Research Institute, a large outpatient clinics in Amsterdam.

The study was approved by the medical ethical committees of the VUmc and Reade and all participants gave written informed consent.

Outcome measures

Filling in questionnaires, sampling of blood, and all measurements, including serum CRP levels, were performed at the start of each study (i.e. the baseline visit), making sure that all measurements were of TNF-naïve patients. When using the term ‘CRP levels’ further on, we are referring to these TNF-naïve CRP levels obtained at the start of each study.

Serum CRP levels were determined on Roche/Hitachi Modular P (at VUmc) and Cobas C (Reade) systems by a particle enhanced immunoturbidimetric assay. Intra- and interassay coefficients of variation for CRP were 1.9% and 3.4% respectively. These measurements were conducted with a detection limit of 1 mg/l. A CRP level ≥ 10 mg/l was considered a parameter of active disease in AS. Several other data collected was: age, sex, HLA-B27 status, BMI, smoking status, disease duration and symptom onset duration, extraspinal manifestations of AS (presence of uveitis, IBD and psoriasis), medication use,

and disease activity scores such as BASDAI, BASFI and global disease activity score (VAS patient).

Genotyping the *CRP* SNPs

Genomic DNA was isolated from peripheral blood by standard procedures. Four common *CRP* gene tagging SNPs with published minor allele frequencies (MAF) of more than 5% that were associated with CRP levels or their proxies were selected on the basis of published work(18;20;21).

Functional polymorphisms in the *CRP* gene region (dbSNP ID) rs2794521 T>C(17;18) and triallelic SNP rs3091244 C>T>A located in the gene promoter(22), as well as rs1800947 G>C, a synonymous coding SNP in exon 2(15) and SNP rs876538 G>A in the 3' flanking region(18;21) were genotyped.

Genotyping was performed with appropriate quality control measures by the Taqman SNP allelic discrimination method with an ABI PRISM 7000 Sequence detection System (Applied Biosystems, Foster City, CA, USA) (details are available from the authors upon request).

Statistics

Statistical analysis was conducted using SPSS V18.0 (SPSS Inc., Chicago, Illinois, USA). On basis of published work, sex, BMI, age, smoking status and NSAID use were selected as possible confounding variables(1;1). CRP levels were correlated with the disease activity status in each patient using BASDAI.

Two linear regression analyses were performed. First, the crude relation between polymorphisms and CRP was analysed and secondly an adjustment was made for sex, BASDAI, BMI, age, smoking status and NSAID use. Because CRP levels were skewed to the right in all linear regression analyses, the natural logarithm of CRP was used as outcome variable. Results were presented as back-transformed geometric means for CRP levels.

An independent samples t-test was used to determine the differences in CRP level between two groups with different disease activity (BASDAI \geq 4 or <4).

Using the linear regression analyses, the effect of the different genotypes and the effects of carrier ship of both the minor and the major alleles on CRP level were tested. For the triallelic SNP rs3091244, the effect of the different genotypes as well as the effects of carrier ship of both minor alleles were analysed. Crude and adjusted logistic regression analyses were performed to determine the effect of SNPs and haplotypes on the dichotomised CRP, with a cutoff point of 10 mg/l. The same potential confounding variables were used in the adjusted model.

The publicly available Haploview version 4.2 software (Cambridge, USA) was applied to estimate pair wise linkage disequilibrium (LD) between the four SNPs examined(23). Haplotypes were constructed to determine the integrated effect of multiple SNPs on CRP levels. Genotype data of each of the four *CRP* SNPs were used to infer the frequency of the haplotypes present in the population using the program Phase v2.1. Only patients with >99% certainty in haplotype estimates were included in the analysis(24).

The same crude and adjusted linear and logistic regression analyses were applied to determine the effect of each haplotype on CRP levels. Additionally, the effects of being homozygous, heterozygous or non-carrier for each haplotype were estimated.

We present otherwise uncorrected two-tailed p values. For all analyses statistical significance was designated as a two-tailed p value less than 0.05.

RESULTS

Patient characteristics

In total 233 patients with near complete data were selected for our study. Excluded were 42 patients because of self-reported non-Caucasian ethnicity and 2 patients who did not fulfil the predefined control criteria for polymorphism and haplotype determination, leaving 189 patients for statistical analysis. The main characteristics of the 189 AS patients under study are listed in Table 1.

Our study population of AS patients encompasses a large variety in disease activity, with a high mean BASDAI of 4.9 (SD 2.2). In total, 47.3% of the patients had a CRP level ≥ 10 mg/l. Mean BASFI was 4.5 (SD 2.7) and mean VASpatient 5.8 (SD 2.6). A high percentage of AS patients (50%) had peripheral arthritis. CRP levels did not significantly differ in patients with extraspinal manifestations such as peripheral arthritis, anterior uveitis and inflammatory bowel disease (data not shown).

Disease activity variables and CRP levels

CRP levels were significantly positively correlated with BASDAI ($p=0.001$), BASFI ($p<0.001$) and VAS ($p<0.001$). BASDAI, BASFI and VAS were all significantly positively associated with each other (all $p<0.001$). CRP values ≥ 10 mg/l were found in 52% of the AS patients with a high disease activity (BASDAI ≥ 4).

Table 1: Characteristics of 189 patients with ankylosing spondylitis.

Characteristics	<i>Means \pm SD or N (cumulative %)</i>
Age (years)	47.8 \pm 11.7
Male	145 (76.7)
Duration of disease (years)	15.0 \pm 10.5
HLA-B27 positivity	185 (86.6)
Smoking status	73 (40.6)
Mean BMI (kg/m ²)	25.4 \pm 4.3
Peripheral arthritis	94 (50.3)
Uveitis	55 (29.3)
Psoriasis	21 (11.2)
IBD	14 (7.4)
Patients on NSAIDs	146 (78.1)
Patients receiving DMARDs	18 (9.6)
Patients on systemic steroids	14 (7.5)
Patients with CRP \geq 10 mg/l	87 (47.3)
BASDAI (0-10)	4.9 \pm 2.2
Patients with BASDAI \geq 4	125 (67.2)
BASFI (0-10)	4.5 \pm 2.7
VAS patient (0-10)	5.8 \pm 2.6

HLA-B27, human leucocyte antigen B27; BMI, body mass index; IBD, inflammatory bowel disease; NSAIDs, non-steroidal anti-inflammatory drugs; DMARDs, disease-modifying antirheumatic drugs; CRP, C-reactive protein; BASDAI, Bath ankylosing spondylitis disease activity index; BASFI, Bath ankylosing spondylitis functional index; VAS, visual analogue scale.

SNPs and CRP levels

The genotype frequencies of all four SNPs were in Hardy-Weinberg equilibrium. Allele frequencies of SNPs rs2794521 (allele T=0.68, allele C=0.32), rs3091244 (C=0.65, T=0.30, A=0.05), rs1800947 (G=0.96, C=0.04) and rs876538 (G=0.77, A=0.23) were in concordance with frequencies found in the Framingham Offspring cohort, a study of 1640 healthy unrelated participants of European descent(21). Of the four SNPs genotyped, rs3091244 was significantly associated with CRP levels. ($p=0.02$) as shown in Table 2.

Patients with the CA genotype showed significantly higher CRP levels when compared to the reference genotype CC ($p=0.02$), and this association remained when adjusted for BASDAI and other confounders in the multivariate regression analyses ($p=0.04$). In addition, AS patients carrying the A-allele of rs3091244 had significantly higher CRP levels compared with patients homozygous for the C-allele when adjusted for all confounders. Rs876538 genotype GA showed nominal evidence for association with CRP levels ($p_{\text{trend}}=0.06$) when compared to genotype GG, but this association did not persist when adjusted in the multivariate linear regression analyses.

Haplotypes and CRP levels

Six haplotypes defined by the four representative SNPs in the *CRP* region were constructed and coded H1-H6 in order of descending frequency (Figure 1A). Linkage disequilibrium between the *CRP* SNPs is shown in Figures 1B and 1C. Haplotype analysis showed that H5 was associated with CRP levels after adjusting for BASDAI and other confounders. A minority of 10.6% of the AS patients was an H5 carrier and these were all heterozygotes. H5 is tagged by the A allele of rs3091244 and AS patients who are carriers of H5 express significantly higher mean serum CRP levels than non-carriers ($p<0.03$),

showing a mean CRP level of 15.4 mg/l in H5 carriers and 8.6 mg/l in H5 non-carriers. This result reflects the same significant association as described above for the A-allele of rs3091244.

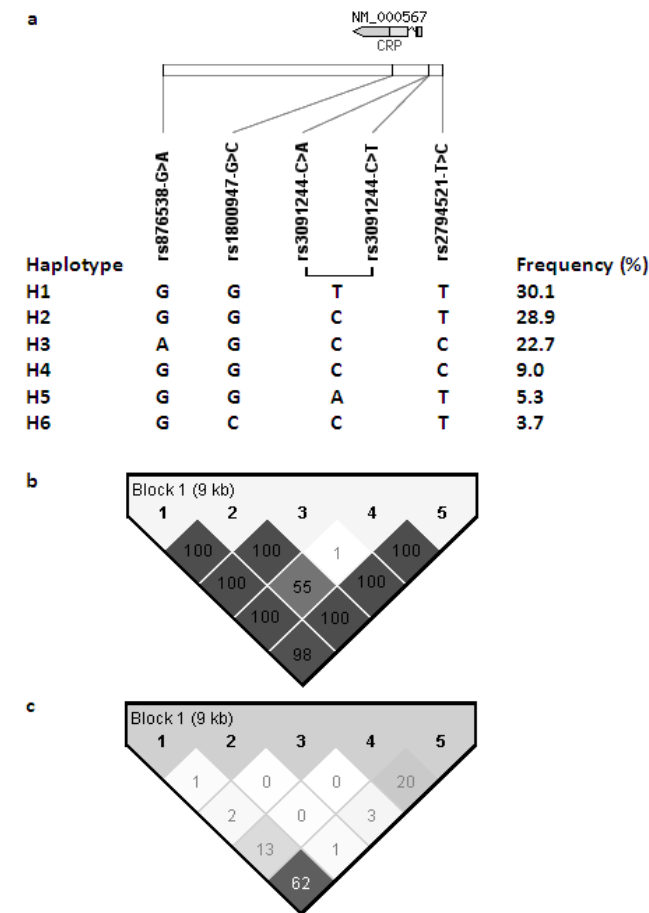
Table 2: Mean serum CRP values in mg/l for CRP SNPs genotypes.

	<i>Frequency, N</i> <i>(cumulative %)</i>	<i>Mean CRP</i>	<i>95% CI</i>	<i>p-Value</i>
<i>Genotypes</i>				
rs2794521 T>C				
TT	91 (48.1)	10.6	ref	ref
CT	76 (40.2)	7.9	5.9– 10.8	0.17
CC	22 (11.6)	8.0	4.5 – 14.2	0.40
rs3091244 C>T>A				
CC	75 (39.7)	8.3	Ref	ref
CT	77 (40.7)	8.9	6.6 – 12.0	0.77
CA	17 (9.0)	18.6	9.9 – 34.8	0.02*
TT	17 (9.0)	8.3	4.4 – 15.5	0.98
TA	3 (1.6)	5.4	1.2 – 24.1	0.58
AA	N.D.	N.D.	N.D.	N.D.
rs1800947 G>C				
GG	175 (92.6)	8.9	ref	ref
GC	14 (7.4)	12.9	6.4 – 25.8	0.31
CC	N.D.	N.D.	N.D.	N.D.
rs876538 G>A				
GG	108 (58.1)	11.0	ref	ref
GA	69 (37.1)	7.5	5.5 – 10.2	0.06
AA	9 (4.8)	5.9	2.5 – 13.9	0.17

CRP values were backtransformed after natural logarithmic correction. N.D.= not detected.

*Significant result.

Figure 1: SNPs in the region of the *CRP* gene at chromosome 1q21-q23 with their pairwise linkage disequilibrium (LD), haplotypes, and haplotype frequencies in ankylosing spondylitis patients.



Legend to Figure

a Location, haplotypes and haplotype frequencies of the four SNPs (with dbSNP accession numbers) in the *CRP* region in 189 AS patients. In addition, one patient carried a recombinant haplotype (not shown).

b LD plot showing pairwise LD between SNPs as $D' \times 100$ (100= maximum possible value) created by the Haploview v4.2 program. [23]

c The r^2 parameter, as determined with the Haploview program, that quantitates intermarker LD is given ($r^2 = 0$ indicates no LD, $r^2 = 100$ indicates complete LD). CRP, C-reactive protein; H, haplotype; SNPs, single-nucleotide polymorphisms.

For clarity, Table 3 shows mean CRP levels in relation to H5 carriage and the BASDAI. Mean CRP level was elevated (≥ 10 mg/l) despite low disease activity (BASDAI <4) in H5 carriers. Table 3 also shows that high disease activity (represented by BASDAI ≥ 4) did correlate with CRP levels in the H5 non-carriers of our study population ($P=0.003$, independent samples t-test).

Table 3: Mean serum CRP level in mg/l for H5 carriers and H5 non-carriers, with high or low disease activity (BASDAI).

	<i>BASDAI ≥ 4 (N=125)</i>	<i>BASDAI < 4 (N=61)</i>
H5 Carrier (N=20)	18.5	11.8
H5 Non-carrier (N=169)	10.5	5.5 *

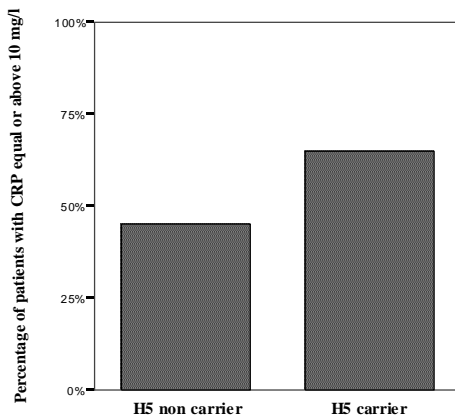
CRP values were backtransformed after natural logarithmic correction.* P=0.003, determined between BASDAI ≥ 4 and < 4 in H5 non-carriers (independent samples t-test). (BASDAI unknown; N=3).

The results of the logistic regression analyses showed that AS patients carrying H5 had a 2.9-fold higher odds (95%CI 1.0 to 8.3; p=0.05) compared with non-carriers to have a CRP value ≥ 10 mg/l, after adjusting for the BASDAI and other potential confounders.

A higher percentage of AS patients carrying H5 (65 %) compared to H5 non-carriers (45%) did express a CRP value ≥ 10 mg/l (Figure 2; p=0.09).

Correction for peripheral arthritis and HLA-B27 status did not change the results.

Figure 2: Percentage of H5 carriers and H5 non-carriers (within each group) that expresses a serum CRP value equal or above 10 mg/l (P=0.09, Chi-square test).



DISCUSSION

In this exploratory study, we investigated the contribution of four *CRP* gene polymorphisms and their haplotypes to CRP levels in AS patients and found a significant correlation between the minor allele A of triallelic SNP rs3091244 and its corresponding haplotype and serum CRP levels after adjustment for disease activity (BASDAI) and other confounders. AS patients with the CA genotype of rs3091244 were associated with significantly higher CRP levels compared with patients homozygous for the major C-allele. Our observation that different genotypes of rs3091244 express different CRP levels is in accordance with other studies(11;18;25).

Moreover, we found that carriers of haplotype 5 (tagged by the A-allele of rs3091244) have a nearly threefold higher odds (OR=2.9) of expressing a CRP value ≥ 10 mg/l, when adjusting for all confounders. Several studies have shown rs3091244 to be one of the most important SNPs in the *CRP* gene region influencing CRP level. Kathiresan et al. showed that rs3091244 explains the largest part of CRP level variance in the 1640 healthy participants of the Framingham Offspring cohort study(21). Other studies also established on basis of haplotype analysis, that rs3091244 was the most important SNP influencing CRP level variation(15;20). Subsequently, in vitro studies by Szalai et al. demonstrated rs3091244 to be functional, by affecting the transcriptional activity of the *CRP* gene(22).

In our study population a relatively small percentage of 10.6% was a carrier of haplotype 5. However, this is an interesting finding as many common (and probably rare) variants genome wide may be influential on elevated CRP level similar to low-range CRP levels(11;12).

We found no correlation between CRP levels and rs2794521, rs1800947 and rs876538.

In previous literature this is confirmed but other authors did find an association between CRP levels and these SNPs (rs2794521(13;15;17;18;26;27), rs800947(13;15;20;21;26;27) and rs876538(18;21;27)).

A possible explanation for the difference between our findings and previous studies might be due to the fact that in most studies, association between *CRP* SNPs and low-range CRP level changes in apparently healthy individuals were studied, whereas our study investigated the genetic influence of common SNPs and haplotypes in the *CRP* gene on CRP ≥ 10 mg/l in AS patients.

CRP and disease activity

Many AS patients do not express clearly elevated CRP levels. This study showed that disease activity (BASDAI) and a certain genotype and haplotype in the *CRP* gene region are both significantly associated with CRP levels in these patients. It also supports the hypothesis that genetic polymorphisms may contribute some patients expressing a lower CRP level, despite high disease activity, as seen in Table 3.

The results show that the CC-genotype of rs3091244 presents with a lower mean CRP level and that carrying the A-allele increases CRP level, as also shown in the haplotype results. AS patients with the CC-genotype in contrast to those carrying the A-allele could show CRP values < 10 mg/l despite high disease activity. This poses a possible explanation why some AS patients do not show an increase in CRP despite high disease activity. This is in concordance with findings by Rhodes et al. in rheumatoid arthritis, who found *CRP* SNPs to influence CRP levels in this disease implicating this effect could influence clinical decision making and lead to suboptimal treatment(25;28).

CRP levels are widely used in AS and recently have been included in the ASDAS, a new disease activity score reported by van der Heijde et al. to be best at discriminating between high and low disease activity at baseline and follow

up and especially being able to correlate with disease activity from a clinical perspective(5). The present study showed that only 52% of AS patients with high disease activity show a corresponding rise in CRP level, showing that genetics influence CRP level. Therefore, one should take into account the genetic influence on CRP levels when assessing disease activity with the ASDAS. This is even more important because clinical decisions, such as starting or stopping treatment with TNF blocking agents, are based on these disease activity parameters. This might lead to under- or overtreatment of AS patients depended on their specific genetic architecture.

Also shown in the results (Table 3) is disease activity (BASDAI above or below 4) significantly influences CRP level in AS patients. This association was not significant in H5-carriers, probably due to small sample size. A recent study by Kiltz et al. also showed that a high BASDAI influences CRP level(29).

A limitation of this study might be the heterogeneous study population, including AS patients who are candidates for anti-TNF treatment and those who do not require this treatment. In order to minimise this difference we have adjusted for all factors that might influence CRP level, especially BASDAI. In addition, although strict selection procedures were applied so that only self-reported Dutch Caucasian patients were included, it can not be ruled out that there are subtle differences in population ancestry and it is not yet established if these results can be generalized to other ethnicities.

Furthermore, CRP measurements were not done with a high sensitivity CRP assay (hsCRP). The more sensitive hsCRP test can measure CRP with the lowest detected level of 0.1 mg/l. However, this is of less importance in this study as higher CRP values are more of interest in the population of AS patients and not the precise differences between low CRP values.

At the time of initiation of this study, the ASDAS was not yet available, therefore BASDAI was used, the best disease activity score at the time, to adjust

for disease activity status in the AS patients. The study is limited by the small sample size. Even with a modestly sized study population of AS patients, we were able to demonstrate significant results. However, further research with a larger study population is required to confirm our results.

To conclude, our data suggest that genetics might play a role in the lack of CRP rise in some AS patients despite high disease activity. The importance of a genetic contribution to CRP levels might be taken into consideration when clinical decisions in AS patients have to be made.

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Discovertebral (Andersson) lesions of the spine in ankylosing spondylitis revisited.

Johannes L. Bron¹, Mirjam K. de Vries², Marieke N. Snieders¹, Irene E. van der Horst-Bruinsma², Barend J. van Royen¹.

Orthopedic Surgery Department¹, Rheumatology Department², VU University Medical Center, Amsterdam, the Netherlands.

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ABSTRACT

A well-known complication in patients with ankylosing spondylitis (AS) is the development of localised vertebral or discovertebral lesions of the spine, which was first described by Andersson in 1937. Since then, many different terms are used in literature to refer to these localised lesions of the spine, including the eponym ‘Andersson lesion’ (AL). The use of different terms reflects an ongoing debate on the exact aetiology of the AL. In the current study, we performed an extensive review of the literature in order to align communication on aetiology, diagnosis and management between treating physicians. AL may result from inflammation or (stress-) fractures of the complete ankylosed spine. There is no evidence for an infectious origin. Regardless of the exact aetiology, a final common pathway exists, in which mechanical stresses prevent the lesion from fusion and provoke the development of pseudarthrosis. The diagnosis of AL is established on conventional radiography, but computed tomography and magnetic resonance imaging both provide additional information. There is no indication for a diagnostic biopsy. Surgical instrumentation and fusion is considered the principle management in symptomatic AL that fails to resolve from a conservative treatment. We advise to use the term Andersson lesion for these spinal lesions in patients with AS.

INTRODUCTION

Ankylosing spondylitis (AS) is a chronic inflammatory disease that primarily affects the spine and sacroiliac joints, causing pain, stiffness and a progressive thoracolumbar kyphotic deformity(1). In the late state of the disease, the spine demonstrates progressive ossification of the annulus fibrosis, anterior longitudinal ligament, apophyseal joints, interspinous and flaval ligaments resulting in a complete ankylosed spine, often referred to as a ‘bamboo

spine’(2). A well-known complication in these patients is the development of a localised vertebral or discovertebral lesions of the spine, which was first described by Andersson in 1937(3). The exact prevalence of discovertebral lesions complicating AS in literature is unknown, but reported prevalences range from 1.5% to over 28%(4-11). This large variation may be explained by the lack of proper diagnostic criteria and the differences in the extent of spinal survey undertaken. Since the study of Andersson, many different terms have been used to refer to these localised lesions of the spine, including the eponym ‘Andersson lesion’ (AL), ‘discovertebral lesion’, ‘vertebral lesion’, ‘destructive vertebral lesion’, ‘spondylodiscitis’, ‘discitis’, ‘diskitis’, ‘sterile diskitis’, ‘pseudarthrosis’ or ‘(stress-) fracture’(4;6;8;10;12-20). The use of so many different terms to describe ALs in patients with AS reflects an ongoing debate on the exact aetiology of these spinal lesions. There also seems to be a discrepancy in the terminology used by the specialists treating patients with AS, most often rheumatologists and orthopaedic surgeons. The aim of this study was to perform an extensive review of the literature of these lesions, in an effort to align communication on aetiology, diagnosis and management between treating physicians.

LITERATURE SEARCH METHODS

A comprehensive search was performed for all scientific literature, written in English and published between 1966 and July 2008 referenced on Medline, concerning discovertebral lesions in AS. The following search string was used: ((ankylosing spondylitis OR spondylitis ankylopoietica OR bechterew OR Marie Strumpell disease OR Marie Struempell disease OR Spondyloarthritis OR bamboo spine) AND (Andersson Lesion OR discovertebral lesion OR vertebral lesion OR destructive lesion OR spondylodiscitis OR discitis OR diskitis OR vertebral osteomyelitis OR pseudoarthrosis OR fracture)) AND

((Humans[Mesh]) AND (English[lang])). The articles were subsequently screened for opinions on aetiology, diagnosis and management. There are no randomised controlled trials available studying patients with ALs. In the current review, we use the term AL to refer to a localised vertebral or discovertebral lesion complicating AS.

DISCUSSION

aetiology

Since the first report by Andersson(3) several aetiologies for the development of ALs in patients with AS have been postulated, including infection, inflammation, trauma and mechanical stress. Because of the radiographic resemblance with osteomyelitis and spondylodiscitis, an infectious origin has been suggested by some authors, but this has never gained much popularity in literature(7;21). Up to now, only two case reports have been reported that might support an infectious aetiology of the AL(19;22). Lohr et al. described a patient with AS, with a history of intravenous drugs abuse, with a T11-T12 spondylodiscitis and a positive culture of *Staphylococcus aureus* in the pleural fluid, urine and one of eight blood cultures(19). The patient was treated by antibiotics, and 6 days later, he underwent a right thoracotomy. Unfortunately, no histology and cultures of the lesion were described after surgery. Nikolaisen et al. described a case with an active inflammatory histological appearance; however, they did not achieve positive cultures by biopsy(22). Subsequently, biopsies have been performed in patients with an AL in many studies, but positive cultures have never been found and tuberculosis was never detected(6;10;11;13;21;22). Furthermore, sedimentation rates in AS patients are often elevated and usually do not further increase when an ALs develops(10). In Table 1, the clinical studies, including at least six patients with AL from the past three decades, are summarised.

Table 1: Overview of studies with at least six AL patients

<i>Author (year)</i>	<i>affiliation</i>	<i>diagnosis</i>	<i>N patients (% of AS patients)</i>	<i>N lesions</i>	<i>Trauma</i>	<i>Posterior elements</i>	<i>(Trans) discal</i>	<i>Trans vertebral</i>	<i>Symptomatic</i>	<i>Level</i>
Cawley (1972)	Reu	Destructive lesion	15 (-)	>18	4	3x fracture	all	-	14 (93%)	>5 T, TL, 8 L
Modena (1978)	Reu	Spondylo-discitis	9 (14%)	>22	n.k.	n.k.	all	-	n.k.	n.k.
Chan (1987)	Rad	Pseudarthrosis	18 (-)	22	n.k.	15x fracture 5x unfused fj	22	-	18 (100%)	4 T, 7 TL, 5 L
Wu (1987), Fang (1988)	Ort	Pseudarthrosis	35 (15,9%)	40	7	34x fracture, 6x unfused fj	37	3	31 (89%)	13 T 9 TL 18 L
Rasker (1996)	Reu	Spondylodiscitis	6 (1.5%)	11	0	n.k.	6	-	6 (100%)	5 T, 1 TL 3 L, 2 LS
Kabasakal (1996)	Reu	Spondylodiscitis	12 (8%)	32	0	0	32	-	2 (17%)	6 T, 4 TL 21 L, 1 LS
Shih (2001)	Rad	Fractures or Pseudarthrosis	16 (-)	16	12	16 x fracture	12	4	16 (100%)	1 C, 8 T 3 TL, 4 L
Langlois (2005)	Reu	Aseptic discitis	14 (18%)	16	1	n.k.	16	-	12 (86%)	1 C, 5 T, 3 TL, 6 L, 1 LS
Chan (2006)	Ort	Pseudarthrosis	30 (-)	30	30	30x fracture	30	-	30 (100%)	All between : T9 and L3
Kim (2007)	Ort	Pseudarthrosis	12 (-)	19	4	n.k.	19	-	7 (58%)	10 T, 1 TL, 7 L, 1 LS

n.k., not known; fj, facet joints; C, cervical; T, thoracic, TL, thoracolumbar; L, lumbar; LS, lumbosacral.

This table demonstrates that most studies either refer to a traumatic/mechanical (pseudarthrosis) or inflammatory (spondylodiscitis) aetiology when describing ALs. Some differences between the studies can be noted; firstly, studies performed by rheumatologists generally refer to spondylodiscitis, whereas orthopaedic surgeons or radiologists always refer to pseudarthrosis. Secondly, when AL is described as spondylodiscitis, there are multiple lesions in most patients, and these lesions are always (trans)discal. Thirdly, when AL is described as pseudarthrosis, usually, a single lesion is observed. The lesion can be transdiscal or transvertebral and is usually accompanied by fractures or non-fusion of the posterior elements. These differences suggest that the ALs that have been studied by the authors might represent a heterogeneous group of lesions. In 1972, Cawley et al. was the first to divide ALs in two different groups: localised lesions and extensive lesions. Localised lesions occurred early in the course of AS, supporting an inflammatory mechanism. These lesions were further subdivided according to their exact location: the discal surface of the vertebral rim or the cartilaginous part of the vertebral endplate. Extensive lesions involved both locations and were exclusively seen in patients with an ankylosed spine(10). Based on radiological features a division in five categories has also been proposed: pseudodystrophic, pseudotuberculous, extensive erosions, bone condensation and isolated narrowing of the intervertebral spaces(8). The first two categories and early erosions should result from an inflammatory process and the remainder and late erosions from mechanical factors. However, none of these theories have become generally accepted.

Inflammation

AS is characterised by spinal inflammation, and it is therefore not surprising that many authors have focussed on an inflammatory aetiology of ALs. Romanus was the first to describe ‘anterior spondylitis’, which comprised

marginal erosions of the anterior vertebral corners related to inflammation of the anterior annulus fibrosus in patients with AS(23). The erosion becomes enclosed by a rim of sclerosis and further healing results in the formation of syndesmophytes finally resulting in a complete ankylosed spinal segment. It has been postulated that AL might be exceptional extensions of this inflammatory process(10;17;23;24). Marsh even documented a symptomatic Romanus lesion roentgenologically, which progressed to a complete destructive lesion within 2 years(25). However, many authors disagree since the Romanus lesion is an enthesitis limited to the junction of the anterior longitudinal ligament and annulus fibrosus(14;26). Furthermore, Romanus lesions usually affect multiple levels and ALs often involves only a single level, indicating that at least additional factors should be involved(8). The localised lesions in the vertebral rim described by Cawley et al.(4) seem to include Romanus lesions, and this might also be the case in other studies. A different theory, for the lesions that mainly involve the vertebral endplates of the intervertebral disc, is the herniation of nucleus material through the endplates(27). This process could be stimulated by vertebral osteoporosis, which is a well-known feature early in the course of AS. Moreover, apophysal joint disease could attribute to the herniation process by increasing forces across the discovertebral junction and stimulating breaks in the endplates and subchondral bone(27). The nucleus pulposus is an avascularised tissue, and contact with the vascularised subchondral bone is suspected to provoke a serious inflammatory response(28). However, there is no evidence thus far to support this hypothesis. A theory that has become more widely accepted combines the inflammatory process with its mechanical effects on the spine. During the course of the disease, the extent of spinal inflammation and spinal fusion is not equally distributed over all vertebral or discovertebral segments. Local areas with increased spinal inflammation and decreased spinal fusion permit an excessive degree of

mobility, resulting in a local non-union of the ankylosed spine(6;15).

ALs show variable results on histological examination (Table 2)(13). Generally, aspecific reactive changes are found with the intervertebral disc being replaced by hypovascular fibrous tissue. The endplates show irregular destruction, extending into the subchondral bone. Fragments of necrotic bone and cartilage are often found across the vertebral border. Mild inflammatory changes may also be present, with infiltration of plasma cells, lymphocytes or, less often, macrophages. Although this infiltration could be a secondary feature, Nikolaisen et al. revealed infiltration with plasma cells (IgA+) and lymphocytes (CD3+) in specimens taken in the early course(22). Later biopsies in the same patients only showed reactive changes with bone formation. According to the authors, these findings support a primary inflammatory origin for ALs(22). Further histological evidence favouring an inflammatory origin is sparse, but several hypotheses have been postulated. Additional factors favouring an inflammatory origin of ALs include the occurrence of lesions early in the stage of AS, before the spine has become completely ankylosed, and the occurrence of multiple lesions within a patient, of asymptomatic lesions and absence of a history of trauma in many patients.

Table 2: Overview of the histological appearances of tissue obtained from ALs reported over the past 20 years

<i>Author (year)</i>	<i>Tissue retrieval</i>	<i>Samples</i>	<i>Description</i>	<i>diagnosis</i>
Fang (1988) and Wu (1987)	Surgical resection	18	Fibrous tissue with a poorly vascularised central zone containing irregular bundles of collagen fibres and aggregates of blood vessels. Irregular destruction of endplates extending into subchondral bone. Fragments of necrotic bone and cartilage across vertebral border. Inflammatory cells generally absent.	PA
Arnold (1989)	Surgical resection	1	Fibrocartilage with low grade inflammatory infiltrate and necrotic bone fragments.	DL
Peh (1993)	Surgical resection	1	Vascularised fibrous tissue and organizing fibrinous exudate adjacent to fragments of bone and cartilage.	PA
Rasker (1996)	Open biopsy	2	Aspecific reactive changes infiltration of lymphocytes, plasma cells and scarce macrophages and destruction of bone.	SD
Lim (1996)	Open biopsy	1	Scanty inflammatory infiltrates.	SD
Petterson (1996)	Open biopsy(1) Fine needle (1)	2	Non-specific chronic inflammation and degenerative changes.	PA
Nikolaisen (2005)	Fine needle aspiration	1	Dense collagenous tissue diffusely infiltrated by regular plasma cells and lymphocytes (CD3 +).	SD
Langlois (2005)	Open biopsy	3	Tissue repair, no evidence of inflammation.	D
Kim (2007)	Surgical resection	19	Hypovascular fibrous tissue with fibrinoid necrosis and chondrodysplasia. Irregular destruction of endplates with sclerotic bony spicules. Fragments of necrotic bone and cartilage within the degenerated fibrotic marrow.	PA

PA, pseudarthrosis; DL, destructive lesion; SD, spondylodiscitis; D, discitis.

Trauma

In many patients, the AL develops after trauma and is associated with a fracture of the spine. The ankylosed and osteoporotic spine in AS patients is prone for fracturing due to loss of spinal mobility(29-34). The bone mineral density in AS patients is often decreased, and approximately half of the patient population can be classified as osteoporotic. This is especially the case in the older patients or patients with long-standing disease. The fractures may occur after a direct trauma or due to chronic mechanical stress.

Acute fractures in AS are most commonly observed near the thoracolumbar junction(35). Especially, in a long ankylosed thoracolumbar kyphotic spinal column, local stresses near the thoracolumbar junction are increased dramatically. Fracturing of the ankylosed spine in AS results in a fracture of both the anterior and posterior part of the vertebral column and may pass through the vertebrae (transvertebral) or more commonly through the calcified disc region (transdiscal)(36). A transvertebral spinal fracture leads to spinal instability, comparable to a flexion distraction fracture or Chance fracture of the vertebral body and the fused posterior facet joints(6;35;37). Traumatic fractures of the cervical spine, however, are also common in patients with AS, but the development of an AL is less frequently observed at this location(32;38). An explanation could be the less severe mechanical loading in the cervical spine compared to the lower spine.

Repeated stress itself may also lead to a fracture of the ankylosed spine, comparable with a stress fracture in long bones(5;6;39). Beside the thoracolumbar junctions, areas that suffer from increased stresses are the levels proximal to the lumbosacral junction, where conditions like disc degeneration and spondylolisthesis occur in the non-rigid spine. Stress fractures may also occur initially in the posterior column prior to the appearance of an anterior

discovertebral lesion(6). The thoracolumbar and lumbar spine have disc spaces most susceptible to shearing or distraction under the effect of gravity in the kyphotic spine. Bone is more susceptible to distraction than to compressive forces. Especially during hyperflexion loads, the posterior elements experience large tension forces and may fail initially(35). A relation between the occurrence of ALs and heavy manual labour has been reported(4;8). In addition, cases have been described of patients who developed a symptomatic AL after improvement of their mobility due to anti-inflammatory treatment or total hip arthroplasty(40;41).

In the complete ankylosed spine, a (stress-) fracture will be the only moving segment between the long lever arms. This is similar to the 'last mobile segment' principle as described earlier and is therefore sometimes referred to as a 'final common pathway'(6;11). Persistent motion at the fracture site may hinder fracture healing and union, resulting in a sclerotic unfused spinal segment. Such a sclerotic spinal lesion can be compared with a hypertrophic pseudarthrosis of a long bone(4;6;11;14;16;42-45).

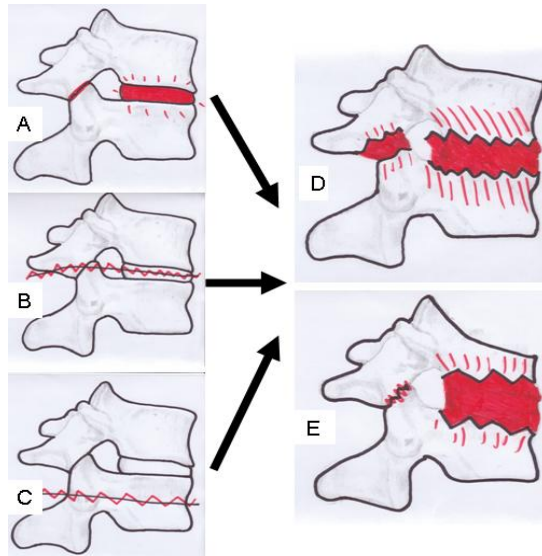
Pseudarthrosis

In an attempt to summarise the available data on aetiology, three different groups may be recognised: firstly, localised lesions that always have an inflammatory origin. Secondly, extensive lesions without fractured posterior elements. These lesions result from a combination of inflammatory and mechanical factors (last mobile segment) and are always transdiscal and associated with unfused facet joints. Thirdly, extensive lesions with fractured posterior elements resulting from mechanical factors ((stress-) fracture) and may be located transdiscal or transvertebral. Lesions from groups 2 and 3 have a final common pathway and will both result in pseudarthrosis with the typical appearance of the AL (Figure 1). It might be questioned whether the first group

of lesions should be considered as ALs since these lesions have a different aetiology, different mechanical consequences and may require different management.

Figure 1: Schematic presentation of the development of discovertebral (Andersson) lesions.

Lesions may originate from inflammation combined with unfused segments (last mobile segment; a), fractures through the ankylosed disc (b) or fractures through the vertebral body (c). Finally, a characteristic Andersson lesion develops, with (e) or without (d) a kyphotic deformity.



History and clinical presentation

Patients with an AL are most commonly middle-aged males (63-86%), known with longstanding AS(8;43;46). However, ALs may occur at any level and during every course of the disease. Cases of patients in whom the AL was the initial sign of AS have been reported(15). The occurrence of an AL has also been described in an 11-year-old female patient(47). The main complaint is progressive localised thoracolumbar pain or sharp localised pain preceded by a (minor) trauma(4;5;48). Patients may also be asymptomatic, and the AL is discovered with routine radiological evaluation of the spine. Sometimes, patients may be unaware or already have forgotten a preceding trauma(8;49).

Occasionally, radiculopathy and neurogenic intermittent claudication due to nerve root or myelum compression may be the presenting symptom(50). The symptoms are aggravated by verticalisation and relieved by lying flat. Clinical investigation may reveal percussion tenderness on the affected spinal level(51). A thoracolumbar kyphosis is often present and may show progression during follow-up. Kauppi stated: “If a bamboo spine starts to bend, something is wrong”. In some patients, multiple lesions may be present or develop simultaneously, which can make diagnosis more difficult. Fever is not a sign of AL and should raise the suspicion for other potential conditions, such as infections. Kabasakal et al. tried to score the severity of the lesion on an empirical 0-10 scale, in which 1 is defined as suspicious change and 10 represents the end stage of the AL(7). However, this scoring system has not been followed in literature.

Radiography

Conventional radiographs are the initial imaging study of choice. Plain radiographs are most reliable to determine the exact level of the lesion when the lowest rib is included in the image. Especially in AS patient presenting with localised pain after a trauma, a high index of suspicion is required(49). Finkelstein et al. showed that a significant part of the spinal fractures is initially missed, allowing progression to pseudarthrosis(16). The AL is revealed by osteolytic destruction with a surrounding zone of reactive sclerosis and vertebral osteophytes(5).

The spinal lesion can be confined to the vertebral body (transvertebral), through the disc space (transdiscal), or both (discovertebral)(36). In transdiscal lesions, the disc space is conspicuously more radiolucent than its neighbour disc spaces which are fused. The lesions may be accompanied by fractures of the posterior elements, which generally can be exposed by lateral radiographs.

An intervertebral vacuum phenomenon may be visible at the side of the lesion, and the disc space can be increased or narrowed. Angular kyphosis with or without spondylolisthesis in the AL is a regular prominent feature. In cases with a severe global thoracolumbar kyphotic deformity, the apex of the kyphosis is most often at the level of the AL (Figures 2A and 3A). Flexion and extension radiographs may show motion in some cases. These radiological features are not pathognomonic for ALs and may also be seen in septic spondylitis or spondylodiscitis. Dihlmann et al. state that AL can be differentiated radiologically from an inflammatory spondylodiscitis by the demonstration of a circumscribed defect in one or two neighbouring vertebral bodies with varying degrees of narrowing of the intervening disc space, angular kyphosis of the affected spinal segment and an area of reactive sclerosis in the vertebral cancellous bone surrounding the defect(24).

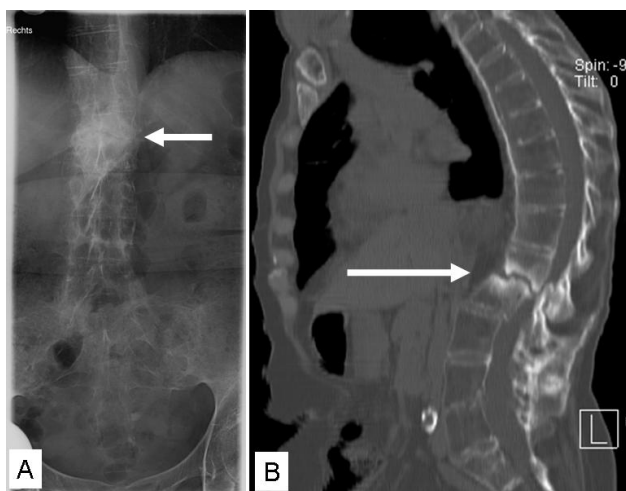
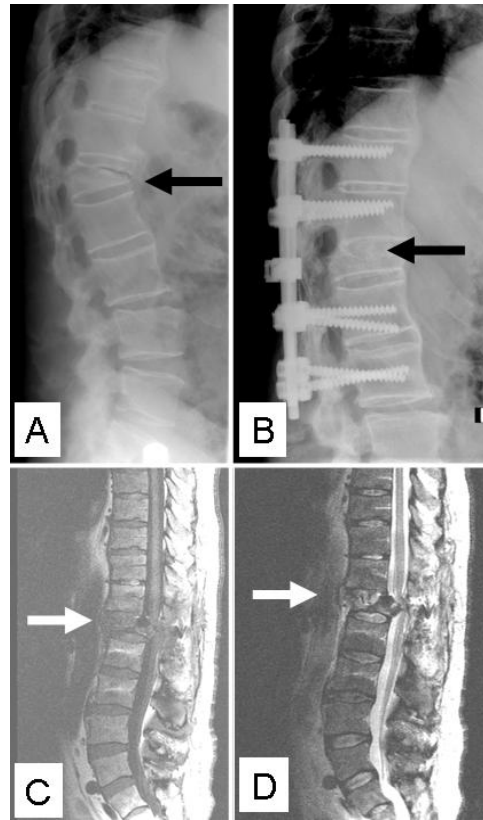


Figure 2: Anteroposterior plain radiograph from an AL at the thoracolumbar junction in a 56-year-old female AS patient (A). A sagittal reconstructed CT image of the same patient shows central osteolysis surrounded by an irregular sclerotic zone (B). The lesions extend into the posterior elements and has resulted in narrowing of the spinal canal.

Figure 3: Lateral plain radiograph with a kyphotic discovertebral AL at the thoracolumbar junction in a 60-year old mal AS patient (A). Radiograph after posterior instrumentation and fusion of the symptomatic AL is also shown (B). T1-(C) and T2-weighted (D) sagittal MR images accurately reveal involvement of the surrounding structures. The lesion has resulted in a severely narrowed spinal canal, with dural compression, that clinically resulted in a postoperative partial peroneal nerve palsy of his right leg.



Computed tomography imaging

Computed tomography (CT) imaging is superior to conventional radiographs in determining the extent of the lesion (Figure 2B). CT imaging of an AL shows irregular vertebral or discovertebral osteolysis with surrounding reactive sclerosis(52). CT can always accurately demonstrate fractures of the posterior elements or non-fusion of the facet joints. In addition to conventional plain radiographs, CT imaging is more sensitive in demonstrating vacuum phenomena and paraspinal swellings. The transversal imaging is used to determine the presence, location, severity and nature of spinal stenosis. Chan et al. describe 18 AS patients with 22 spinal pseudarthrosis, who underwent conventional radiography as well as CT imaging. In 77% of the lesions, CT

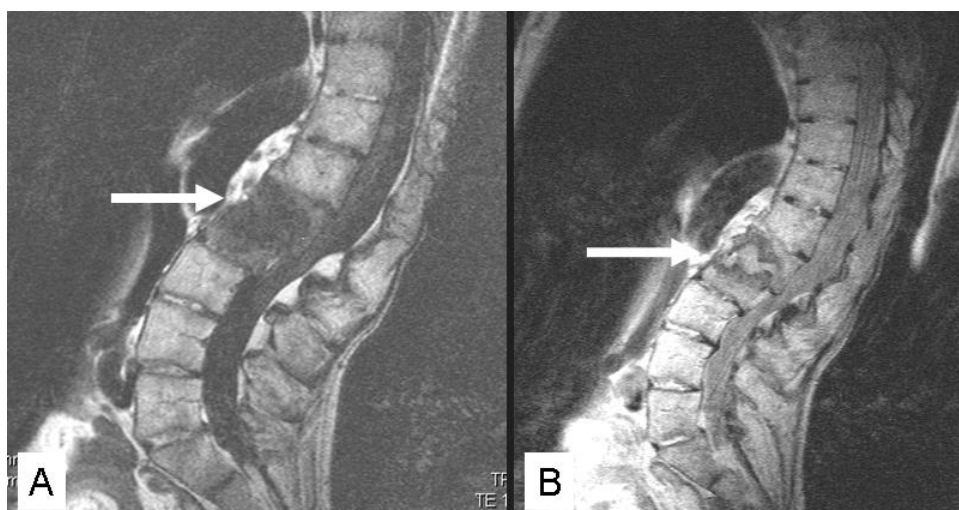
imaging provided data that were missed on conventional radiography. Therefore, CT offers considerable contributory advantages over conventional radiography in diagnosing AL complicating AS(52). However, CT scanning had the disadvantage of irradiation, which should be considered when choosing the appropriate imaging method.

Magnetic resonance imaging

Magnetic resonance imaging (MRI) is considered the best modality in visualising AL with the highest sensitivity(17;39;53). Generally, reduced signal intensity of the disc space and surrounding vertebral bodies and increased signal intensity after enhancement with contrast medium is noticeable on T1-weighted images (Figures 3C and 4A). On T2-weighted images, increased signal intensity in the corresponding area is noticeable (Figures 3D and 4B). In addition, contrast-enhanced fat suppression imaging allows a better differentiation between fat and enhanced lesions. Considering a diagnosed AL of the spine in AS on plain radiographs and CT images, MR images can supplement information on anterior longitudinal ligament disruption, vertebral translation, abnormal dural enhancement, epidural lesions and spinal canal stenosis(39).

In addition, MRI is advised to evaluate spinal canal encroachment and the extend of changes in the dura, spinal cord, nerve roots, soft tissue, and ligaments(17;39;53). A decreased spinal canal can be observed caused by anterior and posterior extradural tissue resulting from hypertrophic ligamentum flavum and facet joints, as well as from hypertrophic callus formation of the anterior and posterior elements of the AL. Differentiation from infectious spondylitis and primary or metastatic tumors of the spine is possible if criteria such as morphology, extension of bone marrow oedema, contrast enhancement and signal intensity in the disc space on T2-weighted and fat suppression sequence are taking in consideration(53;54).

Figure 4: T1-weighted sagittal MRI of a 74-year old AS patient showing an AL at the L1-L2 level with characteristically reduced signal intensity. T2-weighted images reveal a central destructive zone surrounded by an area with reduces signal intensity at both sides.



Bone scintigraphy

The use of both early and late bone scintigraphy can be used to identify AL complicating AS and to differentiate the lesion from infection. However, the literature, which describe the use of bone scintigraphy in patients with AS complicated by AL, is only scarce(9;14;46). Focal areas of increased isotope retention in the late state of AS may identify the AL. In a prospective study of 63 patients with AS, Lentle et al. found evidence of a sterile diskitis in five patients(9). Two of them were not recognised on radiographs and bone scintigraphy led to the diagnosis. Park et al. described 16 patients with a 'spinal pseudarthrosis' complicating AS(46). The spinal lesion was confirmed with bone scintigraphy in all 16 patients. However, Arnold et al. described a case with an AL in longstanding AS in which bone scintigraphy did not demonstrate an increased uptake(14).

Laboratory tests

The role of laboratory tests in patient with an AL is limited. Both erythrocytes sedimentation rate and C-reactive protein levels may be elevated in patients with ALs, but this is of little diagnostic value(4;6;8;22;47). Both parameters are usually more associated with the involvement of peripheral joints(55). White blood count and blood cultures are of no additional diagnostic value.

Histology

There is no indication for a biopsy in patients with AS suspected from an AL. However, tissue for histological examination might be obtained when surgical treatment is performed. The lesions have variable histological appearances discussed earlier (Table 2).

Management

Conservative treatment is often the first step of treatment, but there are no trials available showing its optimal duration. Spontaneous relief of symptoms up to 3 years of conservative management has been reported, and the strategy should be adjusted to each individual patient. In the upper thoracic spine, the presence of an intact sternal-rib complex provides stability and might prevent kyphosis, which was recently described as ‘the fourth column’(56). At the more mobile cervical and thoracolumbar levels, however, conservative management is less efficient. Stabilisation can be obtained by plaster immobilisation or Halo-jacket immobilisation. Despite a normal bone-healing capacity in AS patients, fusion of an extensive AL by plaster immobilisation is not likely(4;6). Even with long-term plaster immobilisation using a thoracolumbosacral orthosis with one hip included, minimal persistent motion at the AL may hinder fracture healing and union. Although plaster immobilisation or Halo-jacket immobilisation has been reported as a success in the treatment of acute fractures in AS(31), there is no

evidence suggesting successful recovery of AL in AS patients after a long time of immobilisation treatment. However, correction of a progressive cervical kyphotic deformity resulting from a cervical fracture can be achieved by immediate halo-jacket immobilisation followed by (gradual) correction by manipulation.

The use of non-steroidal anti-inflammatory drugs during the active phases of the disease forms the mainstay of the pharmacological management of AS. Newer therapies that have been introduced, including anti-tumor necrosis factor therapy such as infliximab, etanercept and adalimumab. There is no evidence that treatment with these drugs is also beneficial in the treatment of symptomatic AL. A rigorous exercise programme, as advocated in the treatment of AS, is contraindicated in patients with AL complicating AS(41).

Surgery

Surgical treatment is indicated in patients with unbearable pain, progression of the symptoms, a progressive kyphotic deformity or neurological deficits. Surgical decompression, stabilisation and fusion form the mainstay of the surgical management of AL complicating AS (Figure 3B). The goal is both to decompress the spinal canal and to restore spinal stability facilitating healing and fusion of the spinal lesion. Numerous surgical techniques have been advocated, including instrumented and non-instrumented stabilisation through anterior, posterior or combined approach(57). The surgical procedure depends on the localisation of the lesion, alignment of the spine, neurological deficit and the location of thecal sac compression(4;6;39). In patients with a (progressive) thoracolumbar kyphotic deformity, posterior correction and fixation of the AL by an anterior opening wedge osteotomy(43) or a posterior transpedicular wedge resection osteotomy(58) may be considered.

CONCLUSIONS

In an attempt to structure the broad spectrum of Andersson lesions complicating AS, a provisory division in localised and extensive lesions can be used. Localised lesions are limited to certain parts of the intervertebral disc and always have an inflammatory origin. Extensive lesions affect the whole disc or vertebral body and may result from both mechanical and inflammatory factors. Aetiologies range from spinal (stress-) fractures to a local delay in the ankylosing process compared to adjacent levels, resulting in the last mobile segment. Regardless of the exact aetiology, mechanical factors in the ankylosed spine will prevent healing of extensive lesions and promote the formation of pseudarthrosis. Initially, plain radiographs should be performed. MRI scanning might be used for further evaluation, and in selected cases, CT scanning can be performed. At this time, bone scanning does not have a place in the analysis of Andersson lesions in AS. There is no consensus in literature regarding the management of these lesions and when surgical intervention should be performed. The mainstay of surgical treatment, however, consists of instrumentation and fusion, with the correction of a kyphotic deformity, when present.

Since radiological appearance, mechanical consequences, prognosis and management in extensive lesions differ from localised lesions, it may be questioned whether the latter should be classified as Andersson lesions. The eponym Andersson lesion should be preserved to extensive lesions, which is actually a spinal pseudarthrosis and the final common pathway of several different aetiologies.

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6.2

Discovertebral (Andersson) lesions in severe ankylosing spondylitis: a study using MRI and conventional radiography.

M. K. de Vries¹, A.S van Drumpt², B. J. van Royen², J.C. van Denderen⁴, R.A. Manoliu³, I. E. van der Horst-Bruinsma¹.

Rheumatology Department¹, Orthopaedic Surgery Department² and Radiology Department³ VU University Medical Center, Rheumatology Department⁴, Jan van Breemen Institute/Reade, Amsterdam, the Netherlands.

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ABSTRACT

Objectives: To investigate the prevalence of Andersson lesions (ALs) in ankylosing spondylitis (AS) patients who will start anti-tumor necrosis factor (TNF) treatment.

Methods: Radiographs and magnetic resonance imaging (MRI) of the spine were performed before therapy with anti-TNF. ALs were defined as discovertebral endplate destructions on MRI, associated with bone marrow edema and fat replacement or sclerosis, a decreased signal on T1, enhancement after contrast administration gadolinium diethylenetriamine pentaacetic acid (Gd-DTPA), and increased signal on T2 and short tau inversion recovery (STIR). Additionally, conventional radiography showed a fracture line, irregular endplates, and increased sclerosis of adjacent vertebral bodies.

Results: Fifty-six AS patients were included, 68% males, mean age of 43 years, and mean disease duration of 11 years. The mean Bath ankylosing spondylitis disease activity index was 6.4, and 24% of all patients had ankylosis. Only one patient showed a discovertebral abnormality with bone marrow edema of more than 50% of the vertebral bodies adjacent to the intervertebral disc of T7/T8 and T9/T10, a hypodense signal area on T1, and a high signal on STIR. Irregular endplates were depicted, and T1 after Gd-DTPA demonstrated high signal intensity around the disc margins. However, no fracture line was visible on conventional radiology, and therefore, this case was not considered to be an AL.

Conclusion: No AL was detected in our AS patients, who were candidates for anti-TNF treatment. One patient showed a discovertebral abnormality on MRI, without a fracture line on conventional radiology. The relative small proportion of patients with a long-established disease might explain this finding for, particularly, an ankylosed spine is prone to develop an AL.

INTRODUCTION

Ankylosing Spondylitis (AS) is a chronic inflammatory rheumatic disease that affects especially males in the second and third decades of life, with a prevalence of 0.5-0.9%(1). The main clinical symptom is inflammatory back pain typically occurring at night and morning stiffness improving after exercise. The pain, stiffness, and limited mobility of the spine can cause severe limitations in daily life activities.

Inflammation in AS usually is localised in the sacroiliac joints and the axial skeleton. The last decade there is an increasing interest in the spinal involvement in AS visible on magnetic resonance imaging (MRI)(2-5). There is a wide range of abnormalities described in the spine of AS patients. Apart from syndesmophytes and ankylosis of the spine resulting in rigidity, in longstanding AS, also focal destructive discovertebral lesions (Andersson lesion (AL)) can occur, also known as AL(6;7). In 1996, Rasker et al. described six cases of spondylodiscitis in AS(8).

Recently, we described a review on ALs, which show disk space narrowing or widening, vertebral bone destruction, a surrounding zone of sclerosis, and local kyphosis at radiographs of the spine (Figure 1)(7).

Figure 1: A Lateral radiograph of the lumbar spine shows an Andersson lesion with extensive bony destruction of the L1-L2 disk with irregular endplates and increased sclerosis of adjacent vertebral bodies (from Van Royen et al. with permission).



One of the causes of an AL is the local inflammation in the spine in combination with a minor trauma. This lesion can be differentiated from the signs of inflammation at the MRI of the spine of active disease in AS, as is often observed in patients who are candidates for tumor necrosis factor (TNF) blocking treatment. An AL might be difficult to detect on clinical symptoms alone because most AS patients suffer from back pain. More important is the fact that an AL requires a different treatment, for instance immobilisation, in contrast with the physical therapy normally prescribed in AS patients.

The aim of this study was to investigate whether in AS patients, who are candidates for treatment with anti-TNF, ALs could be detected.

METHODS

Patients

AS patients were derived from the outpatient clinics of VU University Medical Center (VUmc) and Jan van Breemen Institute (JBI) in Amsterdam. All AS patients who fulfilled the modified New York criteria and met the criteria for starting treatment with TNF blocking agents according to the international ASAS consensus statement(9;10) were included.

Following this ASAS statement, all patients had an active disease as defined by a Bath ankylosing spondylitis disease activity index (BASDAI) ≥ 4 (scale 0-10)(11), had failed to previous therapy with at least two nonsteroidal anti-inflammatory drugs (NSAIDs) and sulphasalazine in case of peripheral arthritis, and should be treated with TNF blocking agents according to an expert. Patients were enrolled consecutively, but mainly because of limited availability of the MR scanner, several patients could not be included. Furthermore, one patient did not fit into the scanner due to severe kyphosis, and several patients suffered from claustrophobia. At baseline, data, conform to daily clinical practice during

treatment with TNF blocking agents, were collected in all patients who gave their written informed consent.

Radiology

Standing anteroposterior and lateral full-length plain films and a MRI of the whole spine were performed before the start of anti-TNF therapy. MRI were obtained at baseline and after 6-24 months of anti-TNF therapy, using T1-weighted spin-echo sequences (T1) before and after administration of the contrast medium gadolinium diethylenetriamine pentaacetic acid (Gd-DTPA), T2-weighted sequences (T2), and fat-suppression sequences (short tau inversion recovery (STIR)). In patients included in the JBI, MRI of the spine was performed in the Amstelland Hospital Amstelveen with a field strength of 0.5 tesla, starting from the third cervical vertebra (C3) and downwards. In patients included in Vumc, MRI of the whole spine was performed with a field strength of 1.5 tesla. Pre- and post- (Gd) T1 TSE, and STIR MRIs were acquired. In Amstelland Hospital Amstelveen, T2 was acquired as well.

The conventional radiographs and MRI were read by two observers, one orthopedic surgeon (BvR) and a radiologist (RM), who were blinded to the status of the subjects. The consensus score was accepted. First, the conventional radiographs were analyzed followed by an assessment of the MRI.

The extent of spinal ankylosis in the total study cohort was estimated by counting extensive syndesmophyte formation or bamboo spine features on conventional radiographs counted by segment. The extent of abnormalities in the discovertebral unit was compared with the same discovertebral unit in different sagittal MR sequences and the other way around.

An AL was defined as an abnormality in the discovertebral junction that shows on MRI diffuse endplate destruction, with associated bone marrow edema and fat replacement or sclerosis(12). The fracture cleft thought the disk space or

vertebra itself is depicted as decreased signal on T1 and increased signal on T2, STIR, and T1 imaging after Gd-DTPA administration, reflecting reactive edema of the fracture. Conventional radiography of the AL must show a fracture line, irregular endplates, and increased sclerosis of adjacent vertebral bodies.

Statistical analysis

This concerns a descriptive study. Continuous variables were reported as the mean \pm SD or, if skewed, as the median (range). Categorical variables were calculated as frequencies and percentages.

RESULTS

In total, 56 AS patients were included, 36 in VUmc and 20 in JBI, and the baseline characteristics of all patients are summarised in Table 1. Patients from the two centers did not differ in sex, age, or disease activity at baseline. Radiographs at baseline were available in 55 of the total 56 patients. MRI was performed at baseline in all 56 patients. In two patients, an alternative MRI procedure was performed due to a severe kyphotic posture. These MR scans were still suitable for the purpose of our study.

Radiology

Systematic examination of conventional radiographs of the cervical, thoracic, and lumbar spine did not reveal any lesions that met the definition of AL. Distinct ankylosis with extensive syndesmophyte formation was seen in 13 of 55 (24%) of the patients determined with conventional radiography of the spine. Spinal ankylosis or bridging syndesmophytes over multiple vertebrae were seen at the cervical, thoracic, and lumbar segment in 16%, 6% and 7% of patients, respectively.

Table 1: Baseline characteristics

	<i>Mean +/- SD</i>	<i>(range)</i>
Number	56	
Age (in years)	43 +/- 10.8	(22 - 73)
Male sex (%)	38 (68)	
Disease duration (in years) ^a	11 +/- 8.7	(1 - 41)
Symptom duration (in years) ^b	21 +/- 11.3	(1 - 49)
BASDAI (0-10) ^c	6.4 +/- 1.4	(4.0 - 9.7)
Tragus-to-wall distance (cm; normal, <15 cm)	16 +/- 6.0	(11 - 44)
Lumbar flexion index (cm; normal, >5 cm)	2.5 +/- 1.2	(0.3 - 5)
Lumbar side flexion (cm; normal, >10 cm)	10 +/- 4.9	(3.8 - 19)
Chest expansion (cm; normal, >5 cm)	3.4 +/- 1.5	(0.5 - 7)

^a Disease duration: mean time between the diagnosis and baseline .

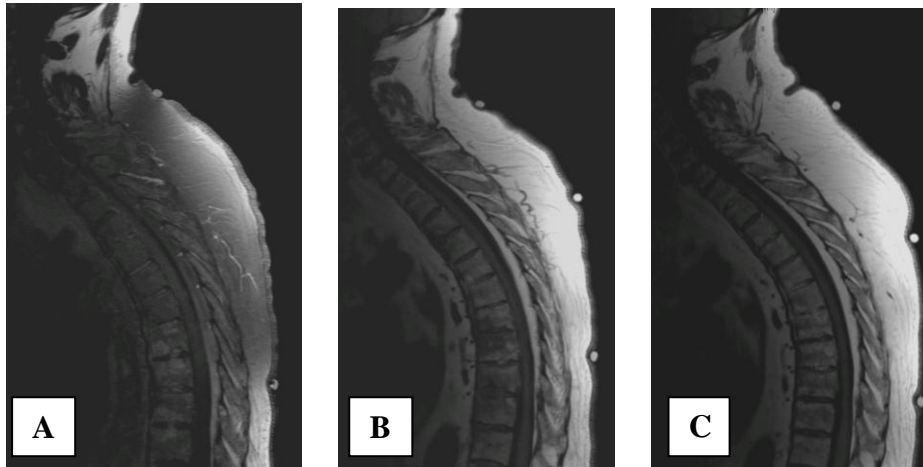
^b Symptom duration: mean time between the first symptoms and baseline.

^c BASDAI : Bath ankylosing spondylitis disease activity index; mean value (11).

Conventional radiographs and MRIs of the whole spine showed no AL according to our definition. However, one patient showed a discovertebral abnormality on MRI. This abnormality showed bone marrow edema of more than 50% of the vertebral bodies adjacent to the intervertebral disk of T7/T8 and T9/T10 (Figure 2), a hypodense signal area on T1 and high signal on STIR. Irregular endplates of the vertebra at these levels were depicted on all sequences. T1 after administration of Gd-DTPA demonstrated high signal intensity around the disk margins of T7/T8 and T9/T10. However, no fracture line was visible on conventional radiology. The clinical characteristics of this patient did not differ from the other patients. He was a male, aged 65 years, with a BASDAI and CRP of 5 and 35 mg/L, respectively, indicative of an active disease. He did not report any specific localising complaints or a preceding (minor) trauma. He had suffered from complaints of low back pain for 40 years and was diagnosed with AS at the age of 51.

Figure 2:

- A. T1-weighted Gd-DTPA post contrast image demonstrated high-signal intensity around the disk margins of T7-T8 and T9-T10.
B. T1-weighted image shows low-signal intensity bone marrow edema in the adjacent vertebral endplates of the T7/T8 and T9/T10 disk spaces.
C. T1-weighted image, obtained 24 months after treatment with anti-TNF agents.



After 24 months treatment with infliximab, T1 weighted images showed a decrease of signs of acute inflammation such as bone marrow edema and maybe even an increase of post-inflammatory fatty bone marrow degeneration around the disk margins. The T1-weighted post-contrast images demonstrated no high signal intensity around the disk margins of T7/T8 and T9/T10 anymore. There were signs of progression of the ankylosis and kyphosis in the thoracic segment after 2 years of follow-up despite anti-TNF treatment. Apart from the focus on ALs, other abnormalities involving the discovertebral unit as described in literature on MRI in AS were encountered. Romanus lesions, which are acute inflammatory lesions at the corners of the endplates of the vertebra, were often detected. These lesions show bone marrow edema in the corners of vertebral endplates and are depicted as hypodense signal areas in T1- and high signal in T2-weighted images(29).

DISCUSSION

In 56 AS patients, who fulfilled the criteria for treatment with TNF blocking agents, only one lesion resembling an AL of the thoracic spine was detected with MRI, but this lesion lacked a fracture line on the conventional radiograph. The low prevalence of ALs was unexpected because this group of AS patients had a relatively high rate of ankylosis of the spine (24%) and signs of active inflammation. This was in concordance with the moderate impairment of the mobility of the spine in these patients. The mean tragus-to-wall distance was 16 cm (normal:<15cm), mean lumbar flexion index was 2.5 cm (normal:>5cm), the mean lumbar side flexion was 10 cm (normal:>10cm) and the mean chest expansion was 3.4 (normal:>5cm)(13). The percentage of ALs as described in literature in this subset of severe AS patients varies between 1% and 28%(14-20).

Many studies report that the presence of an AL is associated with long standing disease in AS and with a rigid, ankylosed spine(21-23), concluding that an AL is a late complication of AS(17). The low prevalence of ALs in our study was probably caused by the relatively short disease duration, with a mean of 11 years and a mean age of 43 years. This could be due to a selection bias, for mostly young patients with an active disease were referred for treatment with TNF blocking agents, whereas the efficacy of TNF blockers in older and more severe cases of AS with complete ankylosis of the spine was still doubtful at the time of the study.

A severe kyphotic deformity and an osteoporotic spine puts a patient at risk of development of a stress fracture in the ankylosed spine(24;25). Our study contains patients with a severe, kyphotic, thoracic spine. However, we did not find any features of stress fractures in this susceptible subgroup of patients, probably due to the small group size.

Another cause of the low prevalence of ALs in this study might be due to the radiographic techniques used to detect them, although the combination of conventional radiographs of the spine and MRI with gadolinium should be sensitive enough. Hermann et al. compared MRI and conventional radiography of spinal changes in patients with spondyloarthritis(21). They concluded that the best way to detect syndesmophytes was through radiography; ankylosis was detected equally well by both radiography and MRI, but for all other lesions, MRI was the preferred method. A disadvantage of conventional radiographs of the spine is the difficult analysis of the vertebrae of the thoracic segment because of the overimposed lung tissue(26), while most disorders of the discovertebral junction occur in the lower part of the thoracic and upper parts of the lumbar spine(27;28). MRI is important for early detection of discovertebral lesions and described as a sensitive method to detect ALs, in particular, after administration of gadolinium(29). Some MRIs in our study showed features of severe degenerative disk disease (DDD), specifically lumbar-sacral. The MRI features of these degenerative abnormalities were sometimes difficult to distinguish from destructive discovertebral lesions as was described by Jevtic et al., who showed that the appearance of an AL can resemble Modic type III degenerative lesion(29). One point to differentiate between AL and DDD in AS is the fact that an AL occurs as a result of inflammation in combination with mechanical stress of the ankylosed spine. Another point is that AL differs from DDD in the localisation, because most AL lesions occur at the level of the cervical and thoracolumbar spine, whereas the DDD predominate at the lumbosacral level(7).

The debate about the prevalence of ALs is hampered by the lack of uniformity in the nomenclature of these lesions. For example, the term AL has been employed for severe destructive discovertebral lesions, but also for minor

abnormalities such as degenerative changes and local inflammation around in the discovertebral junction, as described in our case(7;12).

To conclude, no ALs were detected by using conventional radiographs and MRI in this study in 56 severe AS patients, whereas a higher prevalence was described in literature. The discrepancy might be due to the relatively small group of patient with a short disease duration and young age despite the severe ankylosed spine in our study cohort.

Despite the absence of ALs in our study, we would like to increase the awareness of this complication in AS. In case of a minor spine trauma, a MRI should be combined with the conventional spine radiographs in order to detect this lesion in the stiff and vulnerable spine, which is often osteoporotic as well. The treatment of an AL lesion consists of immobilisation instead of the routinely advised exercise programme or even surgical decompression in case of neurological deficits(7).

In previous studies, there has been no sequential MRI study of the spine in patients with AS that visualizes the evolution from an early discovertebral lesion, as described in our patient, into a severe destructive discovertebral lesion, by some authors called an AL. Thus, it remains unclear whether and how often an abnormality in the discovertebral junction develops into an AL. More research with a long-term follow-up of discovertebral lesions is necessary to clarify the evolution of these lesions.

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7

Improvement of lipid profile is accompanied by atheroprotective alterations in high-density lipoprotein composition upon tumor necrosis factor blockade.

A prospective cohort study in ankylosing spondylitis.

Izhar C. van Eijk¹, Mirjam K. de Vries², J.H.M. Levels³, Mike J.L. Peters², Esther E. Huizer¹, Ben A.C. Dijkmans¹⁻², Irene E. van der Horst-Bruinsma², B.P.C. Hazenberg⁵, Rob J. van de Stadt¹, Gert Jan Wolbink¹⁻⁴, Michael T. Nurmohamed¹.

Rheumatology Department, Jan van Breemen Institute/Reade¹; Rheumatology Department, VU University Medical Center²; Experimental Vascular Medicine Department, Academic Medical Center, University of Amsterdam³; Sanquin Research, Amsterdam⁴; Rheumatology and Clinical Immunology Department, University Medical Center Groningen⁵, the Netherlands.

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ABSTRACT

Objective: Cardiovascular mortality is increased in ankylosing spondylitis (AS), and inflammation plays an important role. Inflammation deteriorates lipid profile and alters high-density lipoprotein cholesterol (HDL-c) composition, reflected by increased concentrations of serum amyloid A (SAA) within the particle. Anti-tumor necrosis factor (anti-TNF) treatment may improve these parameters. We therefore undertook the present study to investigate the effects of etanercept on lipid profile and HDL composition in AS.

Methods: In 92 AS patients, lipid levels and their association with the inflammatory markers C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), and serum amyloid A protein (SAA) were evaluated serially during 3 months of etanercept treatment. HDL composition and its relation to inflammatory markers was determined in a subgroup of patients, using surface-enhanced laser desorption/ionization time-of-flight analysis.

Results: With anti-TNF treatment, levels of all parameters of inflammation decreased significantly, whereas total cholesterol, HDL-c, and apolipoprotein A-I (Apo A-I) levels increased significantly. This resulted in a better total cholesterol:HDL-c ratio (from 3.9 to 3.7) (although the difference was not statistically significant), and an improved Apo B:Apo A-I ratio, which decreased by 7.5% over time ($p=0.008$). In general, increases in levels of all lipid parameters were associated with reductions in inflammatory activity. In addition, SAA was present at high levels within HDL particles from AS patients with increased CRP levels and disappeared during treatment, in parallel with declining plasma levels of SAA.

Conclusion: Our results show for the first time that during anti-TNF therapy of AS, along with favorable changes in the lipid profile, HDL composition is actually altered whereby SAA disappears from the HDL particle, increasing its atheroprotective ability.

These findings demonstrate the importance of understanding the role of functional characteristics of HDL-c in cardiovascular diseases related to chronic inflammatory conditions.

INTRODUCTION

Ankylosing spondylitis (AS) is a chronic inflammatory disease of the sacroiliac joints and spine affecting up to 1% of the population(1). Patients with AS have approximately a 2-fold increased mortality rate compared with general population, which is predominantly due to an increased cardiovascular (CV) risk(2-4). Although specific CV disorders (valvular disease and conduction disturbances) occur more frequently in AS(4;5), accelerated atherosclerotic disease probably contributes to the increased CV risk as well(6;7).

Atherosclerosis is a multifactorial process, but its most well-established risk factor is dyslipidemia. Important prognostic indicators of CV disease are the ratio of total cholesterol to high-density lipoprotein cholesterol (HDL-c) and the ratio of apolipoprotein B (Apo B) to Apo A-I.

Inflammation deteriorates the lipid profile, as characterized by low HDL, total cholesterol, and Apo A-I levels and increased levels of low-density lipoprotein cholesterol (LDL-c), triglycerides, and Apo B. Indeed, several investigators have reported that patients with inflammatory rheumatic diseases have a deteriorated lipid profile(4;6;8-10).

In addition to changes in lipid levels, inflammation can affect HDL qualitatively(11). During inflammation, specific enzyme and protein components of HDL, contributing to its (anti)atherogenic potential, such as serum amyloid A (SAA) and Apo A-I, are modified and may even render it proatherogenic(12). Tumor necrosis factor (TNF) is a pivotal pro-inflammatory cytokine in inflammatory diseases and causes a deterioration of the lipid profile in inflammatory conditions(12).

Treatment with TNF blocking agents, in addition to the known powerful anti-inflammatory effects, may therefore have a beneficial effect on the lipid profile as well as and on HDL composition(13;14).

The current study was designed to investigate whether modulation of inflammatory activity by TNF blockade therapy in patients with active AS is associated with alterations in lipid profile and qualitative changes in HDL composition.

PATIENTS AND METHODS

Patients

Consecutive AS patients attending the outpatient clinics of the Jan van Breemen Institute and VU University Medical Center, in whom etanercept treatment was initiated according to the assessment in ankylosing spondylitis (International Working Group) consensus statement for initiation of anti-TNF treatment(15), were enrolled and followed up prospectively. All patients fulfilled the 1984 modified New York criteria for AS(16) and were treated with subcutaneous etanercept 25 mg twice weekly or 50 mg once weekly. High disease activity was defined as a score of ≥ 4 on the Bath ankylosing spondylitis disease activity index (BASDAI)(17). Patients were included if a baseline serum sample and at least one follow-up serum sample were available. The study was approved by the local medical ethics committee, and all patients provided written informed consent.

Study Design

Data were collected at baseline and after 1 month and 3 months of treatment. During every visit, questionnaires on disease activity (BASDAI) were administered. Total cholesterol, HDL-c, LDL-c, triglycerides, Apo A-I, Apo B, SAA, erythrocyte sedimentation rate (ESR), and C-reactive protein (CRP) were

measured in sera obtained at each time point. Collected sera were stored at -20°C until testing. Commercially available kits were used to measure acute-phase reactants.

Assessment of lipids

Serum total cholesterol and triglycerides were analyzed by an enzymatic method using the appropriate assays (Roche Diagnostics, Almere, The Netherlands), on a Cobas 6000 analyzer (Roche Diagnostics), according to the instructions of the manufacturer. Polyethylene glycol-modified enzymes were used for assessing the HDL-c levels. Apo A-I and Apo B were analyzed by an immunoturbidimetric method, using appropriate assays (Roche Diagnostics). Since we were not able to directly measure LDL-c levels at our laboratory, the Friedewald formula was used, when triglyceride levels were lower than 400 mg/dl, to calculate LDL-c levels, although, in the strictest sense, this formula may not be the most appropriate method for measuring LDL-c in nonfasting samples. The total cholesterol:HDL-c ratio was calculated.

Assessment of inflammation markers

CRP levels were determined using the Roche/Hitachi Cobas 6000 analyzer, based on the principle of particle-enhanced immunologic agglutination (Roche Diagnostics); values of <10 mg/liter were considered normal. High-sensitivity CRP (hsCRP) levels were determined using the Roche/Hitachi Cobas 6000 system, with a detection range of 0.15-20 mg/liter. The test is based on the principle of a particle-enhanced immunoturbidimetric assay; human CRP agglutinates with latex particles coated with anti-CRP monoclonal antibodies. ESR was determined with local measurement techniques (Westergren method); values of <20 mm/hour and <30 mm/hour were considered normal in men and women, respectively. SAA was assessed with an enzyme-linked immunosorbent

assay as described previously(18); values of <4 mg/liter were considered normal.

Preparation of samples

HDL protein profiling was performed as described previously(19). For coating of antibody, a 5 ml mixture containing 2.8 nM anti-Apo A-I monoclonal antibodies, 3 mM ethylenediamine, and 0.1M Na₂SO₄ was added per spot of a PS-20 protein chip, and covalent binding of antibodies through primary amine-epoxide chemistry was achieved by incubating the chip in a humid chamber overnight at 4°C. Excess antibody was removed by one wash with distilled water, and subsequently, free amine binding places were blocked by incubating the chip with 1M Tris buffer (pH 8.4) for 30 minutes at room temperature.

For HDL capture, after mounting of the PS-20 protein chip(s) in a 96-well bioprocessor, 100 µl of plasma aliquots (diluted 1:2 with Tris buffered saline [TBS]) (50 mM Tris [pH 7.4], and 150 mM NaCl) was applied onto each spot and allowed to bind for 2 hours at room temperature on a horizontal shaker. The protein chips were washed 4 times with TBS (5 minutes for each wash), followed by a 2-minute rinse with TBS-Tween (0.005%). A final wash step with HEPES solution (5 mM) was carried out to remove the excess salt. All spots were allowed to dry, and subsequently, 1.2 µl of sinapinic acid (10 mg/ml) in a 50:49.9:0.1% acetonitrile-water-trifluoric acid mix was applied onto each spot. All chips were air-dried and stored at room temperature in the dark until analysis. These measurements were conducted on the same day as the chip processing.

Surface-enhanced laser desorption/ionization time-of-flight (SELDI-TOF) analysis

SELDI-TOF analysis was carried out with a PBS IIc protein chip reader (Ciphergen Biosystems, Fremont, CA), using an automated data collection protocol within the Protein-Chip software (version 3.1). Data were collected up to 200 kd. Laser intensity was set in a range of 190-200 arbitrary units at a sensitivity of 7, and the focus mass was set at 28 kd, specific for anti-Apo A-I capture. Measurement of the spectra was performed with an average of 100 shots at 13 positions per SELDI spot. Calibration was done using a protein calibration chip (Ciphergen). Spectra were normalized on total ion current. Detected peaks having a signal-to-noise ratio of 5 were recognized as significant peaks. Data on the reproducibility of the Seldi technique have been reported previously(19).

Statistical analysis

Data are expressed as the mean \pm SD or the median and interquartile range, as appropriate. The distribution of variables was tested for normality and transformed if necessary. Independent t-tests were used for variables with a normal distribution, and nonparametric tests (Wilcoxon's signed rank test or Mann-Whitney U test) for skewed variables. Pearson's χ^2 test was performed for dichotomous variables. Correlation coefficients (Pearson's) were calculated to evaluate correlations between SAA and lipid levels at baseline.

The generalized estimating equations (GEE) approach was used (a) to analyse longitudinal data on lipids, lipoproteins, and acute-phase reactants measured at 3 different time points (i.e., a longitudinal logistic regression analysis was performed) and (b) to investigate associations between changes in disease activity markers, HDL-c and Apo A-I levels over time.

Absolute and relative changes in lipid levels were calculated in relation to changes in disease activity parameters. Since the total cholesterol:HDL-c ratio and triglyceride levels were not normally distributed, data were analyzed with the logarithms of these values. For clarity, the regression coefficients of these lipids were retransformed to geometric means. Calculations were performed using SPSS 16.0 software. P values less than 0.05 were considered significant.

RESULTS

Characteristics of the patients

A total of 92 consecutive AS patients were enrolled (60 men (65%), 32 women (35%)). The median age was 40.6 years. The mean BASDAI was 6.0, and the median disease duration was 9 years. Ninety-four percent of the patients were taking nonsteroidal anti-inflammatory drugs, 22% were taking concomitant disease-modifying anti-rheumatic drugs, and 8% were known to take statins. During the anti-TNF treatment period of this study, all pharmacologic treatment was unchanged. Baseline characteristics of the patients are shown in Table 1.

Inflammation markers

Concentrations of ESR, CRP and hsCRP were elevated at baseline and declined during treatment ($p < 0.001$) (Table 2). The same was true for SAA, another acute-phase protein, with elevated levels at baseline that decreased significantly after 1 month and remained low and stable thereafter ($p < 0.001$) (Table 2).

At baseline, SAA levels correlated negatively with Apo A-I levels ($r = -0.28$, $p = 0.08$), indicating that higher plasma SAA levels were accompanied by lower Apo A-I levels. Baseline SAA levels did not correlate with HDL-c levels ($r = -0.07$, $p = 0.5$).

Table 1: Baseline characteristics of the 92 AS patients.

<i>Demographic features</i>	
Age, years	43 ± 11.2
Disease duration, median (IQR) years	8.5 (3-18)
N males/females	60/32
HLA-B27 positivity, N (%)†	74 (88)
<i>Disease activity parameters</i>	
ESR, median (IQR) mm/hour	21 (6-38)
CRP, median (IQR) mg/liter	13 (3-35)
hsCRP, median (IQR) mg/liter	11.3 (3.1-33.2)
SAA, median (IQR) mg/liter	5 (2-18)
BASDAI, 0-10 scale	6.0 ± 1.5
<i>Lipids</i>	
Total cholesterol, mmol/liter	4.87 ± 0.9
HDL-c, mmol/liter	1.29 ± 0.4
Total cholesterol: HDL-c ratio, median (IQR)‡	3.89 (3.01-4.90)
LDL-c, mmol/liter	2.92 ± 0.8
Triglycerides, median (IQR) mmol/liter	1.17 (0.89-1.74)
Apo A-I, gm/liter	1.39 ± 0.3
Apo B, gm/liter	0.88 ± 0.2
Apo B:Apo A-I ratio	0.67 ± 0.23

Except when indicated otherwise, values are the mean ± SD. AS, ankylosing spondylitis; IQR, interquartile range; ESR, erythrocyte sedimentation rate; hsCRP, high-sensitivity C-reactive protein; SAA, serum amyloid A; BASDAI, Bath ankylosing spondylitis disease activity index; HDL-c, high-density lipoprotein cholesterol; LDL-c, low-density lipoprotein cholesterol; Apo A-I, apolipoprotein A-I.

†Data not available of 8 patients.

‡Atherogenic index is based on this ratio.

Lipid levels over time

Lipid levels and disease activity parameters in AS patients were measured before and during anti-TNF treatment (Table 2). Total cholesterol, HDL-c, and Apo A-I levels increased significantly during treatment ($p<0.001$, $p<0.001$, and $p=0.004$, respectively). Levels of LDL-c and triglycerides increased slightly during treatment ($p=0.04$ and $p=0.03$ respectively), and Apo B remained stable. The total cholesterol:HDL-c ratio decreased from 3.9 at baseline to 3.7 after 3 months (5% reduction), but this did not reach statistical significance. The Apo B:Apo A-I ratio declined by 7.5%, from 0.67 to 0.62 ($p=0.008$).

Associations between lipids levels and disease activity markers

Since CRP and hsCRP levels were comparable, only CRP was used in the association models. GEE analyses demonstrated several significant associations between lipid levels and disease activity parameters, including CRP, ESR, SAA, and BASDAI, over time, i.e., the degree of disease activity as assessed by the selected disease activity parameters significantly influenced lipid levels. The influence of disease activity parameters on lipid levels is demonstrated by the data shown in Table 3. During the 3-months follow-up period, decreasing levels of CRP, ESR, SAA, and BASDAI levels were significantly associated with increasing total cholesterol levels ($p\leq 0.003$) (with regression coefficients of 0.01, 0.015, 0.006, and 0.063, respectively), increasing HDL-c levels ($p\leq 0.014$) (with regression coefficients of 0.004, 0.005, 0.002, and 0.025, respectively), increasing Apo A-I levels ($p\leq 0.001$) (with regression coefficients of 0.004, 0.005, 0.003, and 0.018, respectively) and decreasing Apo B:Apo A-I ratios ($p<0.01$) (with regression coefficients of -0.002, -0.002, -0.001, and -0.001, respectively). Changes in disease activity parameters were not associated with changes in atherogenic index (total cholesterol:HDL-c ratio).

Table 2: Disease activity parameters and lipid levels in the 92 ankylosing spondylitis patients at baseline, after 1 month and 3 months of etanercept treatment.

	<i>Baseline</i>	<i>1 month</i>	<i>3 months</i>	<i>Regression coefficient (95% CI)</i>	<i>p</i> [†]
Disease activity markers and acute-phase proteins					
CRP, median (IQR) mg/liter	13.0 (3.0 – 35.0)	2.0 (1.0 – 4.0)	2.0 (1.0 – 7.0)	–3.3 (–4.3, –2.6)	<0.001
hsCRP, median (IQR) mg/liter	11.3 (3.1 – 33.2)	1.4 (0.8 – 4.2)	1.6 (0.8 – 5.4)	–4.3 (–6.0, –3.1)	<0.001
ESR, mm/hour	23.5 ± 19.0	8.3 ± 9.5	9.4 ± 11.6	–14.5 (–17.6, –11.4)	<0.001
SAA, median (IQR) mg/liter	4.8 (1.6 – 17.8)	0.9 (0.4 – 2.5)	0.8 (0.2 – 2.2)	–5.4 (–8.0, –3.7)	<0.001
BASDAI, 0-10 scale	6.0 ± 1.5	3.9 ± 2.1	2.8 ± 2.0	–3.2 (–3.6, –2.8)	<0.001
Lipid levels					
Total cholesterol, mmol/liter	4.87 ± 0.88	5.07 ± 0.90	5.10 ± 0.87	0.26 (0.14, 0.39)	<0.001
HDL-c, mmol/liter	1.29 ± 0.42	1.35 ± 0.44	1.42 ± 0.47	0.10 (0.047, 0.15)	<0.001
LDL-c, mmol/liter	2.92 ± 0.79	2.90 ± 0.82	2.93 ± 0.78	0.11 (0.004, 0.22)	0.04
Triglycerides, median (IQR) mmol/liter	1.17 (0.89– 1.74)	1.28 (0.97 – 2.15)	1.37 (0.84 – 2.07)	–0.90 (–0.99, –0.80)	0.03
Apo A-I, gm/liter	1.39 ± 0.30	1.46 ± 0.31	1.48 ± 0.31	0.077 (0.025, 0.13)	0.004
Apo B, gm/liter	0.88 ± 0.21	0.87 ± 0.21	0.86 ± 0.20	–0.002 (–0.026, 0.022)	0.89
Total cholesterol:HDL-c ratio, median (IQR)	3.89 (3.01 – 4.90)	3.85 (2.98–4.89)	3.71 (2.77–4.68)	–0.008 (–0.022, 0.007)	0.32
Apo B:Apo A-I ratio, median (IQR)	0.67 ± 0.23	0.63 ± 0.21	0.62 ± 0.22	–0.035 (–0.061, –0.009)	0.008

Except when indicated otherwise, values are the mean ± SD. Regression coefficients were calculated using generalized estimating equation analysis, with the baseline value as reference. 95% CI = 95% confidence interval (see Table 1 for other definitions). [†]Comparison of 3-month value versus baseline value.

Table 3: Influence of disease activity parameters on lipid levels.

	<i>Absolute change, mmoles/liter</i>	<i>Relative change, %</i>	<i>p</i>
<i>Disease activity parameter (decrease), lipid</i>			
CRP (-10mg/liter)			
Total cholesterol	-0.10	-2.1	<0.001
HDL-c	-0.04	-3.1	<0.001
Apo A-I	-0.04	-2.9	<0.001
Apo B: Apo A-I ratio	0.02	+3.0	<0.001
ESR -10 mm/hour			
Total cholesterol	-0.15	-3.1	<0.001
HDL-c	-0.05	-3.9	<0.001
Apo A-I	-0.05	-3.6	<0.001
Apo B: Apo A-I ratio	0.02	+3.0	<0.001
SAA -10 mg/liter			
Total cholesterol	-0.06	-1.2	0.003
HDL-c	-0.02	-1.6	0.01
Apo A-I	-0.03	-2.2	<0.001
Apo B: Apo A-I ratio	0.01	+1.5	<0.001
BASDAI (-1 point)			
Total cholesterol	-0.06	-1.3	<0.001
HDL-c	-0.03	-1.9	0.001
Apo A-I	-0.02	-1.3	0.001
Apo B: Apo A-I ratio	0.008	+1.2	0.007

The effect of the specified decrease in each disease activity parameters on lipid levels, presented as the absolute change and as the relative change (with the baseline value as reference), was calculated using generalized estimating equation analysis. See Table 1 for definitions.

SELDI-TOF findings

Additional analyses were performed in a subgroup of 10 patients, 5 of whom had high levels of CRP (>30 mg/liter) at baseline and 5 of whom had low levels of CRP (<15 mg/liter) at baseline. After SELDI-TOF analysis, protein spectra from HDL were obtained. Figure 1 shows the HDL profile and Figure 2 shows the plasma SAA levels in 3 representative patients over time. At baseline, a higher density of mass charge (m/z) marker 11,695, which represents SAA, was found in the subgroup of AS patients with high CRP levels. During treatment, all spectra exhibited virtually similar profiles, and m/z marker 11,695 disappeared from HDL as inflammation regressed in the patients in whom CRP levels had been increased at baseline (patients A and B in Figure 1). Moreover, in patient B, a proteolytically generated isoform of SAA appeared to be present; it is known that 1-3 amino acids can be cleaved from either the N- or C-terminus of SAA(20).

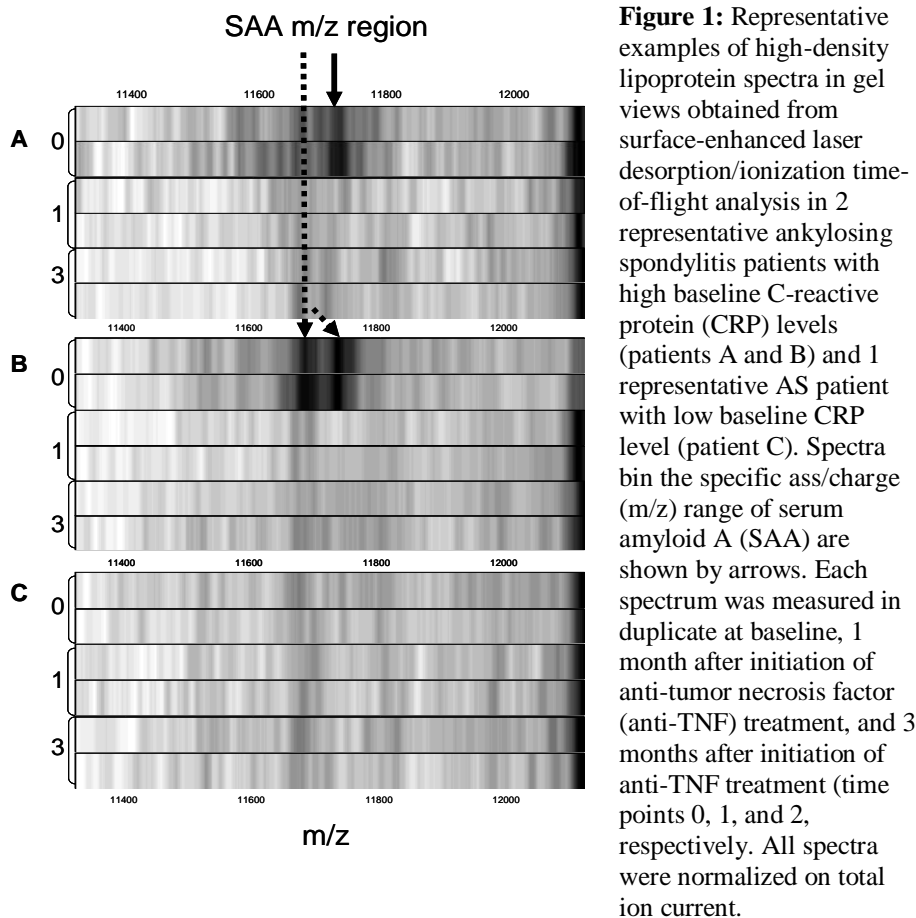
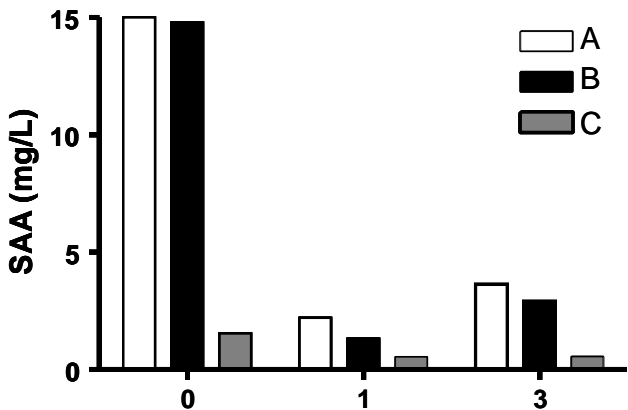


Figure 2: Plasma SAA levels in patients A, B and C at each time point.



DISCUSSION

In the present study, ankylosing spondylitis with high inflammatory activity was characterized by decreased levels of total cholesterol, HDL-c and Apo A-I accompanied by biochemical changes in the HDL particle. Along with improvement of the lipid profile, reflected by increased HDL-c and Apo A-I levels and an improved Apo B:Apo A-I ratio, anti-TNF treatment led to favourable alterations in HDL composition, i.e., diminishing of the SAA concentration within the HDL particles.

This is the first report of a study investigating alterations in apolipoprotein levels in AS patients during anti-TNF treatment. Apo A-I is the major atheroprotective apolipoprotein in the HDL particle, whereas Apo B reflects the total number of potentially atherogenic particles, being present in very low-density lipoprotein, intermediate-density lipoprotein, and LDL.

Comparable with the total cholesterol:HDL-c ratio, the Apo B:Apo A-I ratio has emerged as a very good predictor of future CV events, with the practical advantage that fasting blood samples are not required(21-24). This ratio reflects the balance of cholesterol transport in a simple way. The higher the Apo B:Apo A-I ratio, the more cholesterol is circulating in the plasma compartment, and this cholesterol is likely to be deposited in the arterial wall, causing atherogenesis and risk of CV events. In the AS patients in the present study, the Apo B:Apo A-I ratio was positively associated with disease activity parameters and a 7.5% decrease in this ratio was accomplished during anti-TNF treatment, suggesting a beneficial effect on the risk for CV morbidity and mortality, although, due to the relatively small change in the Apo B:Apo A-I ratio, this should be interpreted with caution.

Anti-TNF treatment resulted in a less atherogenic lipid profile, which is consistent with previous findings(10). Although the observed changes in lipid

levels were small, even these small changes may well have a clinically relevant effect on CV risk, since AS is a chronic inflammatory disease that persists over many years(25). However, beyond focusing solely on HDL-c levels, it seems important to investigate actual HDL composition and thereby its functional characteristics, to learn more about its effects on the vascular system and CV risk.

HDL protein profiling is increasingly being used to determine the biochemical composition of HDL(19;26;27). During acute systemic inflammation HDL becomes pro-inflammatory, loses its protective properties, and can even enhance atherogenesis(12;28). Interestingly, in addition to showing reduced plasma levels of HDL-c during active AS, SELDI-TOF analysis enabled us to demonstrate actual alterations in HDL composition; i.e., in contrast to findings in AS patients with low CRP levels at baseline, in whom virtually no SAA was found on HDL, SAA was markedly present on the surface of HDL in AS patients with increased CRP levels at baseline, but after treatment of these patients to suppress inflammation, the SAA on HDL almost disappeared.

SAA is an acute-phase reactant which is synthesized mainly in the liver in response to pro-inflammatory cytokines such as interleukin-1, interleukin-6, and TNF(29), and elevated levels of SAA are associated with increased CV risk(30). SAA is transported mainly in HDL as an apolipoprotein(31;32). Increased serum SAA levels during the acute-phase response in patients with active AS thus seem to be accompanied by an increased presence of SAA within the HDL particle. Recently, increased SAA within the HDL particle was also found in patients with active Crohn's disease, another chronic inflammatory disease, which is associated with spondylarthropathies including AS(33). This is of interest since it is known that SAA is able to replace anti-atherogenic Apo A-I in the HDL particles, which renders them less protective(34;35). Moreover,

SAA-rich HDL particles are rapidly cleared from plasma, and thus the increase in SAA during inflammation could also contribute to the decrease in total HDL-c concentrations(36). However, other mechanisms may also play a role in decreased HDL-c levels during inflammation as well. It has been suggested that remodelling of HDL through activation of secretory phospholipase A₂ may be an alternate explanation for reduced HDL-c levels during the acute-phase response. Overexpression of this enzyme in mice leads to decreased HDL-c levels and enhanced HDL-c catabolism(28;37-39). In addition, inflammation may convert HDL de novo into a more proatherogenic form by coordinate but inverse transcriptional regulation of SAA and Apo A-I in the liver(29).

This may explain the observed inverse correlation between plasma levels of SAA and Apo A-I, but not between plasma levels of SAA and levels of HDL-c, at baseline. Changes in total cholesterol, HDL-c and Apo A-I levels were significantly inversely associated with changes in levels of disease activity parameters over time, confirming the role of inflammatory activity in lipid profile changes.

In conclusion, findings of the present study demonstrate for the first time that during anti-TNF treatment for AS, along with favourable changes in lipid profile, HDL composition is actually altered, with SAA disappearing from the HDL particle, which rendered it more atheroprotective. Our results highlight the importance of understanding the role of functional characteristics of HDL cholesterol in CV diseases related to chronic inflammatory conditions such as AS.

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Section III

Extraspinal manifestations

8

pANCA, ASCA, and OmpC antibodies in patients with ankylosing spondylitis without inflammatory bowel disease.

Mirjam K. de Vries¹, Ingrid M.W. van Hoogstraten², Adriaan. A. van Bodegraven³, B. Mary E. von Blomberg², Hana Ratnawati⁴, Ben A.C. Dijkmans¹, Irene E. van der Horst-Bruinsma¹.

Departments of Rheumatology¹, Clinical Immunology² and Gastroenterology³, VU University Medical Center, Amsterdam, the Netherlands, Bandung⁴, Indonesia.

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ABSTRACT

Objectives: Patients with ankylosing spondylitis (AS) can suffer concurrently from inflammatory bowel disease (IBD), as ulcerative colitis (UC) or Crohn's disease (CD). Serological markers have been described to diagnose IBD. We investigated IBD serological markers in AS patients without IBD and whether these antibodies enable differentiating patients with AS and IBD from those without IBD.

Methods: Frequencies of perinuclear antineutrophil cytoplasmic antibodies (pANCA), antibodies to the cell-wall mannan of *Saccharomyces cerevisiae* (ASCA), and antibodies to porin protein C of *Escherichia coli* (OmpC) were evaluated in 179 patients: 52 with AS, 50 with UC, 51 with CD, and 26 with IBD and AS. Patient groups were matched for age and sex. All AS patients fulfilled the 1984 modified New York criteria. IBD was ascertained by clinical, endoscopic, and microscopic findings.

Results: In 55% of the AS patients without manifest IBD at least one antibody associated with IBD was observed. pANCA, ASCA (IgA and/or IgG), and OmpC antibodies were found in 21%, 30%, and 19% of the AS patients, respectively. pANCA was more frequently present in AS with concurrent UC than in AS alone (OR 8.2, 95%CI 1.2-55.6), thus being an indicator for UC in AS patients.

Conclusion: Antibodies associated with IBD are detectable in more than half of AS patients without symptoms or signs of IBD. A relatively recent marker in this setting, OmpC antibodies, does not contribute to the differentiation between AS and type of IBD. Presence of pANCA, however, is significantly increased in AS patients who also have UC, and is an indicator to perform endoscopy. These results corroborate a pathophysiological link between AS and IBD.

INTRODUCTION

Several clinical observations imply a common pathogenetic trunk of ankylosing spondylitis (AS) and inflammatory bowel disease (IBD). About 5%-10% of the AS patients have concurrent IBD, either Crohn's disease (CD) or ulcerative colitis (UC)(1-3). In patients with AS without abdominal complaints, 25-49% show microscopic lesions in colon biopsies(4-8). It has been postulated that intestinal inflammation is of (etio)pathogenic importance for development of AS(9). Chronic IBD is usually diagnosed by means of an ileocolonoscopy including histological sampling, which is an invasive, burdensome, patient unfriendly, and costly procedure. Therefore, use of serological markers associated with bowel disease to select AS patients at risk of IBD could be helpful to increase the likelihood of a positive diagnosis following an invasive procedure. In IBD, several of such markers have been proposed to help identify patients at risk for either CD or CU(10-13).

Previous research in our center showed that in UC perinuclear antineutrophil cytoplasmic antibodies (pANCA) had a sensitivity of 63% and a specificity of 86%(12). In CD, antiglycan antibodies to the cell-wall mannan of *Saccharomyces cerevisiae* (ASCA) had a sensitivity of 72% and a specificity of 82%(10;12). Other investigators have reported similar results(10). An even higher specificity is obtained by combining ASCA and pANCA(12). Antibodies to porin protein C of *Escherichia coli* (OmpC antibodies) are also observed in CD, present in about 40% of patients(10;14). These serological markers, in particular ASCA and OmpC antibodies, are rarely positive (<5%) in healthy controls(10;14-16).

Generally, IBD and AS respond well to treatment with tumor necrosis factor (TNF) blocking agents, although several TNF blockers do not show therapeutic efficacy in IBD. In AS the registered TNF blocking agents infliximab,

adalimumab, and etanercept show effectiveness in up to 70% of patients, but in IBD, etanercept appeared to be ineffective(17). As most studies are performed in CD, only limited data are available on effectiveness of anti-TNF in UC, but recent studies indicated that infliximab has (limited) efficacy in UC(18). Identification of AS patients with concurrent IBD could therefore be important in an optimal choice of (costly) biological therapy.

The primary objective of our study was to investigate which serological markers of IBD are elevated in patients with AS who have no symptoms or signs of IBD and whether presence of these antibodies in AS with concurrent IBD enables differentiation from AS without IBD.

METHODS

Patients

Consecutive patients with AS and/or IBD were included after matching for age and sex. These patients were invited to participate if their visits and demographic and clinical data were registered and sera were stored for analysis in our biobank.

One hundred and eighty-two patients were enrolled in the study: 52 AS patients without symptoms or signs of IBD, 52 patients with UC, 52 patients with CD and 26 patients with concurrent IBD and AS. Matching was performed to prevent a bias of the serological testing due to age or sex differences between groups. Selected patients regularly visited gastroenterology and rheumatology outpatient departments of VU University Medical Center, Amsterdam, The Netherlands, a third-line referral center. Diagnosis of AS was based on the 1984 modified New York criteria(19). AS patients with persistent gastrointestinal (GI) complaints or diarrhoea, unexplained weight loss, or iron-deficiency anaemia were referred to the Department of Gastroenterology to exclude GI disease, and IBD in particular. All AS patients with persistent abdominal

complaints underwent endoscopies (N=10). IBD patients with inflammatory-like back or joint pain were referred to the Department of Rheumatology, where patients were analysed for the presence of sacroiliitis. Patients were consequently moved into the correct patient group and data analysis was performed after final diagnosis, including AS only, AS plus CD, AS plus UC, or CD or UC without AS.

Diagnosis of CD and UC was based on standard endoscopic, histological, and radiographic features(20). Disease localization and behavior were documented according to the Vienna classification(21).

Outcome measures

Assessment of serological markers

Most AS patients, and a large number of IBD patients, received anti-TNF therapy, but all blood samples used for this study were obtained before therapy was started. Examinations were performed in a blinded fashion, without knowledge of patients' clinical information. Sera were frozen at -80°C until testing.

pANCA

Determination of pANCA was performed by an in-house indirect immunofluorescence technique using ethanol-fixed human peripheral blood neutrophils as substrate as described by Linskens, et al.(12). Sera were incubated at 1:20 and 1:80 dilutions. Readout was done by visual scoring by 2 readers independently in 3 categories: negative (negative or borderline immune fluorescence at dilution 1:20), weakly positive (positive at 1:20 but negative at 1:80 serum dilutions), or strongly positive (titer \geq 1:80). To investigate the possibility of a false-positive pANCA due to antinuclear antibodies (ANA) additional testing with HEp-2000[®] slides (ImmunoConcepts, BMD, Sacramento,

CA, USA) was performed in case of a (weakly) positive pANCA test result. Cutoff for positivity was set at 1:80.

ASCA

ASCA-IgA and ASCA-IgG were determined using commercial enzyme-linked immunosorbent assay (ELISA) kits (Inova, Uniprom Diagnostics BV, Krimpen aan de IJssel, The Netherlands).

The antigen consisted of phosphopeptidomannan (PPM) extracted from *S. cerevisiae*. ASCA ELISA were performed according to the manufacturer's instructions; results were expressed as arbitrary units with a cutoff for positivity at 25 U/ml as advised by the manufacturer, resulting in a sensitivity for CD of 49% for ASCA-IgA and 74% for ASCA-IgG, and in 1.4% versus 4% positive in healthy controls. Serum was considered positive if either IgA or IgG or both were positive. Serum was considered negative if both ASCA-IgA and ASCA-IgG were negative.

OmpC antibodies

IgA antibodies to bacterial OmpC were detected according to the manufacturer's protocol (Inova, Uniprom Diagnostics BV). The results are presented as arbitrary units with a cutoff for positivity at 25 U/ml.

Serological profiles

The usual UC serological profile was arbitrarily defined as pANCA+ in 1:80, ASCA (IgA or IgG) <25U/ml, and OmpC antibodies <25U/ml.

The classical CD serological profile was arbitrarily defined as: pANCA negative or positive in 1:20, ASCA (IgA or IgG) >25U/ml or OmpC antibodies >25U/ml.

Statistical analysis

Frequencies and percentages of pANCA, ANA, ASCA-IgA and ASCA-IgG, and OmpC antibodies were calculated in each group of patients. χ^2 analysis or Fisher's test was performed to examine dichotomous variables.

Logistic regression analyses were conducted to examine associations between serological markers and serological profiles. The influence of confounders on this relation was investigated. The following variables were investigated: age, sex, and presence of HLA-B27. Statistical analyses were performed with SPSS 15.0 software. The threshold for statistical significance was set at $p < 0.05$.

RESULTS

We recruited 182 patients for the study, 52 patients with AS without IBD, 26 with concurrent AS and IBD (11 AS plus UC, 15 AS plus CD); and 50 patients with UC and 51 with CD, both without AS. Two UC patients and 1 CD patient were excluded as serum samples were unavailable. Demographic data of these groups were similar (Table 1). Nonsteroidal anti-inflammatory drugs (NSAIDs) were used daily by 67 (86%) of all AS patients, immunosuppressive drugs were used in a minority, and TNF blocking agents were started in a majority of AS and CD patients (Table 1).

As expected, within the IBD group, pANCA was more frequently seen in UC ($p < 0.0001$), whereas ASCA and OmpC antibodies were more frequent in CD ($p < 0.0001$, $p = 0.006$, respectively; Table 2). These results were comparable with our previous evaluation of pANCA and ASCA in patients with UC and CD (12). In the overlap group with concurrent AS and IBD, pANCA was more frequent in patients with UC as well ($p = 0.024$); as well, ASCA was more frequently observed in CD although this did not reach statistical significance. OmpC antibodies did not differ between the 2 AS groups.

Table 1: Patient characteristics (N= 179) and clinical variables.

<i>Group</i>	<i>AS</i> <i>N=52</i>	<i>UC</i> <i>N=50</i>	<i>CD</i> <i>N=51</i>	<i>AS+UC</i> <i>N=11</i>	<i>AS+CD</i> <i>N=15</i>
<i>Characteristic</i>					
Male, N (%)	29 (56)	23 (46)	21 (41)	5 (46)	6 (40)
Age, yrs, mean (SD)	41 (12)	37 (11)	36 (13)	43 (11)	38 (12)
Caucasian, N (%)	46 (89)	35 (78)	36 (78)	9 (82)	12 (80)
HLA-B27 positivity, N (%)	42 (84)			5 (83)	10 (67)
Peripheral arthritis, N (%)	21 (40)			1 (9)	1 (7)
Uveitis, N (%)	16 (31)			1 (9)	0 (0)
CRP at baseline	11 (2.5-163)			9 (2.5-37)	29 (2.5-294)
Duration of IBD, yrs, mean (SD)	NA	8 (7)	11 (9)	9(8)	15 (12)
BASDAI at baseline, mean (SD)	6.3 (1.6)	NA	NA	6.2 (1.0)	6.0 (1.9)
Vienna classification					
Age: onset <40 yrs, N (%)			43 (88)		8 (89)
Behaviour					
Inflammatory, N (%)			17 (35)		6 (67)
Stricturing, N (%)			13 (27)		3 (33)
Perforating, N (%)			19 (39)		0 (0)
Localization					
Terminal ileum, N (%)			10 (20)		0 (0)
Colon, N (%)			14 (29)		4 (44)
Ileocolonic, N (%)			24 (49)		5 (56)
Upper GI, N (%)			1 (2)		0 (0)
Immunosuppressive,N (%)	3 (6)	5 (10)	4 (8)	3 (27)	4 (27)
Anti-TNF, N (%)	51 (98)	1 (2)	23 (46)	3 (27)	11 (73)

AS, ankylosing spondylitis; UC, ulcerative colitis; CD, Crohn's disease; BASDAI, Bath ankylosing spondylitis disease activity index (0-10). NA: not applicable

Table 2: Antibodies present in serum of patients (N=179) with ankylosing spondylitis (AS) , ulcerative colitis (UC), Crohn's disease (CD), AS with concurrent UC, and AS with concurrent CD.

	<i>AS</i>	<i>UC</i>	<i>CD</i>	<i>AS+UC</i>	<i>AS+CD</i>
pANCA IgG (%)	11 (21)	33 (66)	8 (16)	7 (64)	3 (20)
ASCA IgA (%)	10 (19)	4 (8)	19 (37)	1 (9)	4 (27)
ASCA IgG (%)	4 (8)	5 (10)	27 (53)	1 (9)	2 (13)
OmpC antibodies IgA (%)	10 (19)	6 (12)	18 (35)	3 (27)	5 (33)
UC profile	8 (17)	25 (50)	4 (8)	6 (67)	0 (0)
CD profile	13 (28)	3 (6)	27 (54)	2 (20)	4 (27)

pANCA, perinuclear antineutrophil cytoplasmic antibodies, positive: titer $\geq 1:80$; ANA, antinuclear antibodies, tested if pANCA-positive; ASCA, antiglycan antibodies to the cell wall mannan of *Saccharomyces cerevisiae*, positive: >25 U/ml; OmpC antibodies, antibodies to porin protein C of *Escherichia coli*: >25 U/ml.

Prevalences of serological markers in AS without IBD

Presence of any out of the 4 serological markers was observed in 55% of AS patients without symptoms or signs of IBD. ASCA (IgA and/or IgG) and OmpC antibodies, markers of CD, were found to be positive in 30% and 19%, respectively, of AS patients without IBD, versus the reported prevalence of 5% or lower in healthy controls(10). pANCA was found in 21% of the AS patients without IBD.

Differences between patient groups

Differences in frequencies of pANCA between patient groups were not due to ANA: a positive ANA was found in about 25% of each group (results not shown). pANCA was statistically significant more frequent in AS with concurrent UC than in AS alone (OR 8.2, 95% CI 1.2-55.6, adjusted for the presence of HLA-B27), thus being an indicator for UC in AS. Moreover, the serological profile for UC differentiated between AS patients with concurrent

UC versus AS alone. In patients with CD no serological marker or profile differentiated between AS with concurrent CD and AS alone. No association was found between presence of HLA-B27 and presence of IBD in AS patients (data not shown).

Colonoscopy

All AS patients without IBD who had persistent abdominal complaints underwent endoscopies and IBD was excluded. One out of 10 patients had signs of microscopic chronic inflammation in the cecum, but there were not enough arguments for IBD, according to the usual definition(20). The 10 patients did not have more frequent peripheral arthritis, higher C-reactive protein, or positive serology (pANCA, ASCA-IgA or ASCA-IgG, anti-OmpC), nor did they have higher levels of anti-OmpC compared to the AS patients without GI complaints. Hence, this particular cohort/group could not be distinguished by serological profile (data not shown).

DISCUSSION

Our study reports on the prevalence of serological markers associated with IBD (pANCA, ASCA, and OmpC antibodies) in patients with AS. For proper evaluation, 3 groups of patients with chronic inflammatory diseases were included: patients with AS, with IBD, and with concurrent AS and IBD.

All the serological markers measured were frequently observed in AS patients: pANCA, ASCA-IgA, and ASCA-IgG antibodies in 21%, 19%, and 8% of the 52 AS patients, respectively. Further, we demonstrated for the first time that OmpC antibodies are highly prevalent in AS patients (19%). These markers, notably ASCA and OmpC antibodies, rarely occur in healthy controls(10). The frequencies of serological markers in AS patients with concurrent UC or CD, except for OmpC antibodies, were comparable with our reference findings in

UC or CD alone. Our findings are in concordance with reports showing increased prevalence of pANCA (22), in particular ASCA, in patients with AS(15;22;23). Positive ASCA-IgA was found in 19% of AS patients without IBD and in 27% in the 15 patients with AS and CD, which is not in concordance with the study of Hoffman, et al.(23), who found no difference among positive ASCA tests in a small series of AS patients with IBD (N=6) and without IBD (N=12). The observed prevalence for the various markers in these series, however, was higher than that reported in a recent study by Mundwiler, et al.(24).

pANCA was statistically significantly more frequently present in AS patients with concurrent UC than in AS alone, with an OR of 8.2 (95% CI 1.2-55.6). pANCA thus might be a valuable tool to screen AS patients with abdominal complaints: if pANCA is present, performance of an endoscopy is indicated.

Despite this high prevalence of IBD markers in AS patients, our study might be flawed due to the fact that invasive colonoscopy was performed in 10 of 52 AS patients (20%). Indeed, colonoscopy was performed only in patients with persistent GI complaints, because the a priori chance of detecting IBD in asymptomatic patients is considered to be very low. However, even in the AS patients with intestinal symptoms, and therefore a relatively 'high-risk' group, the frequency and level of antibodies did not differ from the asymptomatic patients. The explanation for the increased levels of IBD markers in AS is in accord with the studies performed by Mielants, et al.(25), who found a high number of mucosal lesions in asymptomatic AS patients, in particular in the terminal ileum, without convincing macroscopic signs of inflammation by microscopic examination. Intestinal damage (and increased intestinal leakage) itself may be associated with increased IBD markers, but our study was not designed to confirm this association. Interpretation is thwarted due to group sizes. It is interesting, however, that 6.5 % of the cases reported by Mielants

developed IBD after 2-9 years of follow-up(8).

Involvement of the GI tract in AS can be interpreted in 3 ways: as an aberrant immune response following a GI infection, as part of an inflammatory disease sharing a common genetic background, or as a result of intestinal leakage due to treatment with NSAID. As a first hypothesis, AS is induced by a pathogenic microbial, such as *Klebsiella*(26;27), similarly to the onset of another form of spondylarthropathy, i.e., reactive arthritis, which is related to the culprit intestinal pathogen (*Salmonella* species), which induces an aberrant immune response. However, previously no correlation was found between the presence of ASCA in serum and *Saccharomyces* in the intestine(28).

A second hypothesis assumes that a common genetic background in combination with external factors triggers an (auto) immune response against different organs, such as intestines, spine, joints, eyes, or skin(1;29).

Corroborating this hypothesis, extraspinal manifestations are frequently observed in AS patients, such as those with uveitis, which also occurs in IBD.

The third hypothesis is that serological IBD markers, notably ASCA and OmpC antibodies, reflect intestinal damage. Fitting in with this hypothesis is that in celiac disease, which is associated with damage to the intestinal barrier, the prevalence of ASCA is increased(30). Moreover, chronic use of (maximum dosages of) NSAID may result in increased intestinal leakage, leading to an (auto) immune response against intestinal antigens(31). From this perspective one might expect higher prevalence of ASCA in other patient groups taking NSAID on a regular basis, such as patients with rheumatoid arthritis, but these data have not been reported.

Together, IBD-associated serological markers are insufficient sensitive for identifying IBD in AS, but presence of pANCA might differentiate AS patients with concurrent UC from patients without UC, and these patients are candidates for colonoscopy.

We demonstrate that the presence of IBD-associated markers in AS patients is indicative that AS and IBD share a similar pathophysiological origin. These findings apply to AS patients with and without proven IBD since serum markers were also found in AS patients without (symptoms of) IBD.

Prospective follow-up of patients with AS and positive IBD serology markers in comparison with seronegative patients might shed new light on this discussion and contribute to the decision on whether to perform ileocolonoscopy, and on which TNF blocking agents might be most effective, as some (e.g. etanercept) are not effective in colitis(16).

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9

The relationship between disease-related characteristics and conduction disturbances in ankylosing spondylitis.

Vincent K. Dik¹, Mike J.L. Peters^{1,2}, Pieter A. Dijkmans¹, Mignon A.C. van der Weijden¹, Mirjam K. de Vries¹, Ben A.C. Dijkmans^{1,2}, Irene E. van der Horst-Bruinsma^{1,2}, Michael T. Nurmohamed^{1,2}.

Rheumatology Department VU University Medical Center¹, Rheumatology Department Jan van Breemen Institute/Reade², Amsterdam, The Netherlands.

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ABSTRACT

Objectives: Ankylosing spondylitis (AS) is associated with an increased cardiovascular (CV) risk. Conduction disturbances (CD) may explain the CV burden, as they are independently associated with cardiac disease. The aim of this study was (a) to determine the prevalence of CD in AS, and (b) to evaluate the relation between CD and demographic and AS-related characteristics.

Methods: A rheumatological evaluation assessing demographic and AS-related characteristics and a resting standard 12-lead electrocardiogram (ECG) were performed in 131 consecutive AS patients.

Results: A first-degree atrioventricular (AV) block was found in six (4.6%) patients. One (0.8%) patient suffered from a complete right bundle branch block (RBBB) and one (0.8%) patient had a left anterior hemiblock. A prolonged QRS (pQRS) interval was observed in 38 (29.2%) patients, including those with a complete or incomplete BBB. Age, disease duration, and body mass index (BMI) were significantly associated with the PR interval, and male sex, disease duration, and the Bath ankylosing spondylitis metrology index (BASMI) with QRS interval. In multivariate analyses, disease duration remained independently associated with both PR and QRS intervals.

Conclusion: Intraventricular CD is highly prevalent in AS, particularly in patients with long-standing disease. Further research is needed to determine whether intraventricular CD contribute to the increased CV risk and long-term CV mortality in AS.

INTRODUCTION

Spondyloarthropathies are a group of common inflammatory rheumatic disorders, with ankylosing spondylitis (AS) as the major subtype.

The prevalence of AS in the population is in the range 0.1-1.4%, and AS occurs predominantly in early adulthood in men. AS is characterized primarily by sacroiliitis and inflammation of the (lumbar) spine, causing inflammatory back pain. In addition, extra-spinal manifestations such as uveitis, psoriasis and inflammatory bowel disease are common.

Human leukocyte antigen (HLA)-B27 is present in approximately 90-95% of patients with AS, and is thought to play an important role in the pathophysiology of AS(1).

AS is, in accordance with other rheumatic diseases, associated with an increased risk of developing cardiovascular (CV) complications, including atherosclerotic disease, aortic root and valve regurgitation, and a decreased myocardial performance(2;3). Inflammation and/or fibrosis may play an important role in affecting the aortic root and mitral valve, which may extend to the membranous portion of the interventricular septum, the atrioventricular (AV) node, and both bundle branches, causing conduction disturbances (CD)(4-6). It has also been recognized that HLA-B27 is associated with CD(7).

Several studies have reported a higher CV mortality in AS, which may be explained partially by a higher prevalence of CD. However, literature on this topic is sparse and not well defined(2;8). Therefore, the aim of this study was (a) to determine the prevalence of CD in AS, and (b) to evaluate the relation between CD and demographic and AS-related characteristics.

METHODS

Patients

Patients attended the rheumatology outpatient clinics. A total of 131 consecutive AS patients derived from two different cohorts were included. Forty-four consecutive patients were included from an 'early AS-cohort', which comprised AS patients with a diagnosis duration of less than 2 years, and 87 patients were included from an 'AS cohort', which comprised AS patients with an active disease (Bath ankylosing spondylitis disease activity index ≥ 4 (BASDAI)), for which treatment with tumor necrosis factor (TNF) blocking agents was initiated. Baseline data were used for the current analyses.

Rheumatological evaluation

Disease duration was defined as the moment of diagnosis until the moment of entry in this study, in years. Disease activity was determined by using BASDAI (scale 0-10) and Bath ankylosing spondylitis functional index (BASFI, scale 0-10). Physical function and spinal mobility were tested by using Bath ankylosing spondylitis metrology index (BASMI, scale 0-10).

Blood samples were taken to assess HLA-B27 status, and the inflammatory markers erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP). In addition, body mass index (BMI) and medication use were determined.

Electrocardiography

Standard 12-lead electrocardiograms (ECGs) were recorded at 25 mm/s paper speed. One cardiologist, unaware of the patients' health status, analysed all ECGs according to a standardized protocol (heart rate, rhythm, electrical axis, and conduction intervals). The PR interval was defined as prolonged when ≥ 200 ms (pPR) and the threshold for a prolonged QRS (pQRS) interval was set at ≥ 100 ms (9).

Statistics

The distribution of variables was tested for normality and expressed as mean (\pm standard deviation) or median (interquartile range) where appropriate. Univariate and multivariate linear regression analyses were performed to determine the association between demographic and AS-related characteristics on the outcome variables PR and QRS intervals. Additional analyses were performed to investigate the influence of anti-rheumatic, pulmonary, and/ or CV treatment on the PR and QRS intervals. Statistical analyses were performed using SPSS version 14.0. The threshold for statistical significance was set at $p < 0.05$.

RESULTS

Baseline characteristics

One patient was diagnosed with the Brugada syndrome. This patient was excluded, leaving 130 AS patients for further analyses. The demographic and AS-related characteristics of these patients are summarized in Table 1. None of the patients were using TNF blocking agents.

Table 1: Demographic and AS-related characteristics in all patients (N=130).

Age (years)	39.4 ± 10.4
Disease duration (years)	3.9 (1.1 - 10.9)
Male	63.8
HLA-B27 positive	86.2
BMI	25.1 ± 4.0
ESR (mm/h)	12.0 (6.0 - 30.0)
CRP (mg/L)	10.0 (2.0 - 28.3)
BASDAI	5.9 (4.1 - 7.3)
BASMI	3.0 (1.0 - 6.0)
BASFI	5.9 (3.1 - 7.4)
Beta blocker	7.0
Beta2 agonist	4.7
NSAIDs	90.7
DMARDs	10.9

BMI, body mass index; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; BASDAI, Bath ankylosing spondylitis disease activity index; BASMI, Bath ankylosing spondylitis metrology index; BASFI, Bath ankylosing spondylitis functional index; NSAID, non-steroidal anti-inflammatory drug; DMARD, disease-modifying anti-rheumatic drug. Values are given as mean ± standard deviation, median (interquartile range), or percentage.

Arrhythmias and CD

Electrocardiographic data are shown in Table 2. Mean PR and QRS intervals were 151 ± 21 and 94 ± 10 ms, respectively. The mean heart rate was 65 ± 12 beats/min, and 39 (30.0%) patients were bradycardic (heart rate <60 beats/min). A first-degree AV block (i.e. a PR interval ≥ 200 ms) was found in six (4.6%) patients and no second- or third-degree AV blocks were observed.

An incomplete right bundle branch block (RBBB) was observed in 11 (8.5%) patients and one (0.8%) patient suffered from a complete RBBB. None of our patients had a complete or incomplete left bundle branch block (LBBB) and only one (0.8%) patient had a left anterior hemiblock. A pQRS interval (i.e. a QRS interval ≥ 100 ms) was present in 38 (29.2%) patients, including those with a complete or an incomplete BBB.

Table 2: Arrhythmias and conduction disturbances in all patients (N=130).

Heart rate (beats/min)	65 ± 12
Bradycardia (<60 beats/min)	39 (30.0)
PR interval (ms)	151 ± 21
QRS interval (ms)	94 ± 10
First-degree AV block	6 (4.6)
Second-/third-degree AV block	0 (0.0)
Prolonged QRS interval	38 (29.2)
Incomplete RBBB	11 (8.5)
Complete RBBB	1 (0.8)
Incomplete LBBB	0 (0.0)
Complete LBBB	0 (0.0)
Left anterior hemiblock	1 (0.8)

AV, atrioventricular; RBBB, right bundle branch block; LBBB, left bundle branch block. Values are given as mean \pm standard deviation or number (percentage).

Conduction intervals, demographic and AS-related characteristics

Univariate linear regression analyses revealed a significant association between age, disease duration, BMI, and PR interval (Table 3). In addition, QRS interval was significantly associated with disease duration, male sex, and BASMI. A trend was found suggesting an association between pQRS interval and presence of HLA-B27. Moreover, when focusing on male patients only, the prevalence of a pQRS appeared to be significantly higher in HLA-B27-positive patients than in HLA-B27-negative patients (44.3% vs. 10.0%, $p=0.045$). In addition, HLA-B27-positive male patients more often had a pQRS than HLA-B27-positive female patients (44.3% vs. 11.1%, $p=0.001$). Multivariate regression analyses revealed an independent association between age, disease duration, and PR interval, and also an independent association between disease duration, male sex, and QRS-interval. These results were not influenced by anti-rheumatic, pulmonary, and/or cardioprotective treatment.

Table 3: Univariate associations between conduction intervals and demographic and ankylosing spondylitis-related characteristics.

	<i>PR interval</i>		<i>QRS interval</i>	
<i>Demographic and AS-related characteristics</i>	<i>B (95% CI)</i>	<i>p-value</i>	<i>B (95% CI)</i>	<i>p-value</i>
Age (years)	0.60 (0.26 - 0.93)	0.001	0.06 (-0.10 to 0.23)	0.456
Disease duration (years)	0.75 (0.38 - 1.11)	<0.001	0.32 (0.14 - 0.49)	<0.001
Male	3.28 (-4.29 to 10.85)	0.393	8.84 (5.58 - 12.10)	<0.001
HLA-B27 positive	-2.94 (-13.94 to 8.05)	0.597	4.85 (-0.32 to 10.01)	0.066
BMI (kg/m ²)	1.23 (0.23 - 2.22)	0.016	0.35 (-0.10 to 0.79)	0.124
ESR (mm/h)	0.06 (-0.12 to 0.24)	0.487	0.02 (-0.06 to 0.10)	0.621
CRP (mg/L)	0.02 (-0.13 to 0.17)	0.801	0.05 (-0.02 to 0.12)	0.149
BASDAI	-0.17 (-1.92 to 1.57)	0.845	-0.41 (-1.24 to 0.43)	0.334
BASMI	0.62 (-0.85 to 2.09)	0.404	1.02 (0.33 - 1.70)	0.004
BASFI	-0.21 (-1.64 to 1.21)	0.769	0.11 (-0.57 to 0.80)	0.745

B, regression coefficient; CI, confidence interval; BMI, body mass index; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; BASDAI, Bath ankylosing spondylitis disease activity index; BASMI, Bath ankylosing spondylitis metrology index; BASFI, Bath ankylosing spondylitis functional index.

DISCUSSION

The most important finding of this study is the high prevalence of first-degree AV blocks and pQRS intervals (4.6% and 29.2%, respectively). In addition, we observed that both PR and QRS intervals were independently associated with disease duration, suggesting that the inflammatory burden affects the conduction system of the heart.

It is being increasingly recognised that AS is associated with an increased CV risk. The high prevalence of prolonged conduction intervals may be a potential explanation for the CV burden among AS patients, as CD are independently associated with an increased long-term CV mortality(10). The lack of a control group in the present study is a limitation and comparison of the prevalence of CD with that observed in other cohorts needs to be made with caution. Nevertheless, the prevalence of CD among healthy controls reported in large-scale observational studies is considerably lower. For example, in a large study with 122,043 aircrew men, aged between 16 and 50 years, the prevalence of first-degree AV blocks was 0.65%(11). In addition, the prevalence of pQRS (QRS interval ≥ 100 ms) in the Framingham study was 6.1% in male and 2.4% in female subjects(12;13). It should be noted that, although a high prevalence of pQRS was observed, only two (1.5%) AS patients were diagnosed with a complete BBB. However, many patients already had either a pPR or a pQRS at a relatively young age, and in light of the independent association between the QRS interval and disease duration, it could be argued that the number of complete BBBs will increase over time. Previous observations from studies investigating CD and their association with AS-related characteristics are contradictory. Some state that CD in AS patients occur as an intermittent and inflammatory process, while others believe that CD occur particularly in patients with long-standing and more severe disease(14;15).

Our data are inconsistent with the first hypothesis, as we observed no association between the inflammatory markers (i.e., ESR and CRP) and the duration of the QRS interval, but are consistent with the latter, as the duration of the QRS interval appeared to be significantly associated with disease duration and BASMI.

Inflammation and fibrosis located in the membranous part of the interventricular septum may cause intraventricular CD in AS(5;6). However, most previous studies indicate that CD in AS are mainly located in the AV node, which might be the consequence of an abnormality of the AV node artery, rather than septal fibrosis(5). Our observations suggest that the inflammatory burden might induce septal inflammation and fibrosis as well as abnormalities of the AV node artery, causing supra- and intraventricular CD in AS. It should be noted that information about the presence or absence of ischaemic or valvular heart disease was lacking, which prevented us from evaluating the potential contribution of these diseases towards the development of CD.

HLA-B27 is another potential risk factor for the development of CD. In fact, it has been demonstrated that, even in the absence of rheumatic manifestations such as AS, HLA-B27 is strongly associated with CD in males(5;7).

In agreement with these observations, we observed that a pQRS was more prevalent in HLA-B27-positive male patients than in HLA-B27-negative male patients, supporting a role for HLA-B27 in the development of CD in male AS patients.

In conclusion, our observations strengthen the need for further (prospective) research to unravel the pathophysiological mechanisms responsible for the increased prevalence of CD and to investigate the extent to which CD may lead to clinically relevant CV disease in AS.

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General Discussion and Summary

GENERAL DISCUSSION and SUMMARY

TNF blocking agents were introduced in 1998, after decades of limited possibilities for treatment of AS patients, such as exercises and NSAID's. The use of TNF blocking agents has diminished the disease burden of many AS patients dramatically. Patients who did not have any other treatment options before, received a treatment which made their lives almost back to normal again without pain and stiffness. The majority of AS patients (60%) respond very well, but many others, unfortunately, do not. There is no difference in response rate between the various TNF blocking agents: infliximab, adalimumab and etanercept(1-3). In primarily non responsive cases, this lack of response may be due to the fact that disease activity is monitored with a questionnaire, the BASDAI, which is a validated outcome parameter for AS, but which has the disadvantage of subjectivity without a direct relation with inflammatory parameters, such as acute phase reactants (ESR e.g.). A relatively high pain score, due to enthesitis or pain caused by structural deformities, for instance, may raise the BASDAI, even when there is little inflammation in the spine or sacroiliac joints.

On the other hand, patients who initially respond very well, might lose this responsiveness during continuous treatment with TNF blockers. Part of the mechanism behind this secondary non-response was unravelled by measurement of serum levels of anti-TNF and the detection of antibodies against these drugs, as described in **Section I**.

Immunogenicity

In AS patients, inefficacy of infliximab or adalimumab correlated with the presence of low serum trough infliximab or adalimumab levels and the presence of antibodies against infliximab or adalimumab (**Chapter 2 and 3**), whereas

cases with good clinical responses showed the opposite (high serum trough levels and no antibodies against anti-TNF). Moreover, our data have demonstrated that development of anti-infliximab antibodies can precede an infusion reaction. The mechanism of the decrease in efficacy can be explained by lower serum trough infliximab or adalimumab levels, caused by neutralizing antibodies against the idiotype of infliximab and adalimumab, and enhanced clearance due to immune complex formation of antibodies against biologicals and the biologicals(4). Our hypothesis is that when drug levels decrease below a critical limit, the foreign protein might no longer be tolerated and might become immunogenic. The starting dose of infliximab (5 mg/kg) is higher than that of RA (3 mg/kg), which might suppress immunogenic reactions(5). Often, the infliximab dose is increased in AS when responsiveness subsides, but reasons for dose escalation in AS have not yet been well defined. More research is needed to assess whether anti-TNF levels can be used for to determine the optimum dose of anti-TNF in AS in daily clinical practice.

Another option for trying to prevent ineffectiveness caused by antibody formation is the concomitant administration of other immunosuppressive drugs. In contrast with treatment of RA, anti-TNF in AS is given without methotrexate. This may be an explanation for the higher incidence of anti-infliximab and anti-adalimumab formation in AS. In Crohn's disease and in RA, the concomitant use of immunosuppressive drugs or corticosteroids has proven to decrease antibody formation against infliximab(5;6). It has to be investigated whether coadministration of immunosuppressive drugs (such as methotrexate) inhibits antibody formation, because despite the use of immunosuppressive drugs in RA, anti-infliximab and anti-adalimumab formation occurs in around 17% of RA patients treated with adalimumab(7) compared with 31% of AS patients. Moreover, methotrexate has so far not proven to be effective on clinical symptoms in AS in contrast to RA, so the benefits of an additional drug that

might slow down or inhibit formation of anti-TNF antibodies should be weighed against the side effects of this drug.

Another TNF blocking agent, etanercept, does not seem to have high immunogenic properties. In **Chapter 4**, no correlation was found between etanercept levels, formation of antibodies against etanercept and clinical response. All patients had detectable serum levels of etanercept and no antibodies against etanercept were found during 6 months of treatment.

Interestingly, there seemed to be no difference in mean etanercept levels between responders and primary non-responders. These findings are in contrast with our previous studies with infliximab(8;9) and adalimumab in AS(8).

Therefore, this study seems to confirm the hypothesis that etanercept is less immunogenic than other TNF blocking agents, although the duration of this study was too short to exclude anti-etanercept formation after a longer period of therapy with etanercept.

Several arguments are in favour of the hypothesis that etanercept shows less immunogenicity than other TNF-inhibitors. Firstly, etanercept has a less immunogenic structure compared with other TNF blocking agents. Etanercept is a dimeric fusion protein consisting of two TNF receptors, linked to the Fc portion of an immunoglobulin (Ig)G1. Only the fusion part of the molecule may contain immunogenic epitopes. Infliximab is a chimerical monoclonal IgG1 antibody against TNF, partly consisting of murine protein. Adalimumab is a fully human monoclonal antibody against TNF. These monoclonal antibodies have more epitopes within the variable region of the antibody to which an immune response can be directed. Secondly, major fluctuations in serum levels may precipitate an immune response and the development of antibodies against the TNF blocking agent. This is mainly the case in treatment with infliximab, which is administered once every six to eight weeks. Treatment with etanercept, however, produces stable levels between two injections and it is dosed more

frequently (once a week). Thirdly, there may be different mechanisms for non-response, for example caused by inadequate blocking of TNF. This can be caused by enhanced clearance or as a result of inadequate dosing. A dose-response relation of etanercept in AS has not been investigated systematically. We are very interested to learn to what extent new TNF blocking agents such as golimumab and certolizumab pegol will prove to be immunogenic. Golimumab is a fully human monoclonal IgG1 against TNF, which is administered once a month, whereas certolizumab has a totally different structure, containing a Fab' fragment of monoclonal IgG1 anti-TNF antibody linked to polyethylene glycol which is dosed every other week.

As discussed in **Section I** there is some doubt about whether validated patient questionnaires, such as the BASDAI, are the most optimal outcome parameters to measure disease activity in AS. Therefore some studies were performed to explore other biomarkers (**Section II**) of disease activity.

Inflammation in AS does not only cause symptoms such as pain and stiffness, but also increases comorbidity due to cardiovascular disease by accelerating the process of atherosclerosis. Next to biomarkers of disease activity, studies were performed to examine biomarkers of cardiovascular disease in AS. The second chapter in **Section II** contains a review on Andersson lesions and its prevalence in our AS cohort. It is important to consider the presence of such a lesion, as specifically AS patients with an ankylosed spine, who have had a (minor) trauma, have an increased risk at an AL.

Biomarkers of disease activity: inflammatory markers

The study in **Chapter 5.1**, in our large prospective cohort of AS patients, has demonstrated that a combination of elevated baseline levels of CRP and SAA can be a valuable tool in the selection of AS patients who are likely to respond to treatment with anti-TNF. Moreover, inflammatory markers, CRP and SAA in

particular, seem to be useful in monitoring the level of inflammation in patients with AS who are treated with etanercept or infliximab.

Most AS patients showed a significant decrease of several inflammatory markers upon treatment with anti-TNF. In some cases, a secondary increase of these inflammatory markers was seen, which may have been caused by a concurrent infection or inadequate therapeutic levels. Although about 68% of the AS patients in this study had elevated inflammatory markers before the start of anti-TNF therapy, it is known that normal inflammatory markers do not necessarily indicate a low disease activity in AS(10). That is why inflammatory markers were, until recently, not implemented for assessment of disease activity or response to treatment, which is in contrast to RA. This study supports the previous data that raised inflammatory markers are indicative of active disease in AS. It seems useful to add decrease of inflammatory markers to response criteria for continuation of anti-TNF treatment in AS patients showing elevated inflammatory markers at baseline. In 2009, a new ASAS endorsed disease activity score for AS was developed, the ASDAS(11). This tool for assessment of disease activity in AS was derived in analogy with the DAS used in RA. The ASDAS includes the domains of back pain, duration of morning stiffness, patient's global assessment, peripheral pain or swelling and CRP or ESR. This score promises to improve comparison of individual patients' disease activity, and the individual score gives a more reliable reflection of the disease activity at that particular moment.

Despite the fact that ESR showed the strongest association with the BASDAI over time, we consider ESR the least suitable parameter for inclusion in the ASDAS, because in our study, it had no additional value and the half-life of this inflammatory marker was too long for early detection of changes.

Since anti-TNF therapy is very expensive and not without risks, it is of great importance to identify patients who are likely to respond to this type of drug. At

this moment, we believe that inflammatory markers can be very useful predictors for a good response, but a raise of inflammatory markers should not be mandatory for allowing AS patients to be treated with anti-TNF, because patients with normal baseline levels of CRP and SAA may respond to anti-TNF therapy as well.

Additionally, in **Chapter 5.2**, we investigated whether CRP levels, the important acute phase reactant, are influenced by common single-nucleotide polymorphisms (SNPs) and haplotypes in the *CRP* gene. We saw that genotypes and the haplotype tagged by allele A of rs3091244 associated with high CRP levels, independent of BASDAI and other confounders. Therefore, the carrying of distinct genetic variants might explain the lack of elevated CRP levels despite high disease activity in some AS patients. This observation can be important for interpreting disease activity scores that incorporate CRP levels, such as ASDAS.

Discovertebral lesions in AS

Apart from reversible inflammatory signs of the pelvis and spine, visible on MRI, chronic structural lesions can occur in AS, such as the development of localised vertebral or discovertebral lesions of the spine, first described by Andersson in 1937(16) (**Chapter 6**). We conducted an extensive review of literature in order to align communication on aetiology, diagnosis and management between treating physicians. In an attempt to structure the broad spectrum of Andersson lesions (ALs) complicating AS, a provisory division in localised and extensive lesions can be used. Localised lesions are limited to certain parts of the intervertebral disk and they always have an inflammatory origin. Extensive lesions affect the whole disk or vertebral body and may be caused by both mechanical and inflammatory factors. Aetiologies range from spinal (stress-) fractures to a local delay in the ankylosing process compared to

adjacent levels, resulting in the last mobile segment. There is no evidence for an infectious origin. Regardless of the exact aetiology, mechanical factors in the ankylosed spine will prevent the healing of extensive lesions and promote the formation of pseudarthrosis. The diagnosis of AL is established with conventional radiography, but computed tomography and magnetic resonance imaging will both provide additional information. Surgical instrumentation and fusion with the correction of a kyphotic deformity, when present, is considered to be the principle treatment of a symptomatic AL that does not respond to conservative treatment(17;18). The eponym Andersson lesion should be preserved to extensive lesions, which is actually a spinal pseudarthrosis and the final common pathway of several different aetiologies.

In our AS patients, with a high disease activity, only one lesion resembling an AL of the thoracic spine was detected with MRI before start of the therapy, but this lesion lacked a fracture line on conventional radiograph. The low prevalence of ALs was unexpected because this group of AS patients had a relatively high rate of ankylosis of the spine and signs of active inflammation. The percentage of ALs, as described in literature in this subset of severe AS patients, varies between 1-28%(19-25). The low prevalence of ALs in our study was probably caused by the relatively short disease duration and small sample size. This could be due to a selection bias mainly for young patients with an active disease were referred for treatment with TNF blocking agents, whereas the efficacy of TNF blockers in older and more severe cases of AS with complete ankylosis of the spine was still doubtful at the time of this study.

Despite the absence of ALs in our study we would like to increase the awareness of this complication in AS. In case of a minor spine trauma an MRI should be combined with conventional spine radiographs in order to detect this lesion in the stiff and vulnerable spine, which is often osteoporotic as well(26;27). In previous studies, there has been no sequential MRI study of the

spine in patients with AS that visualizes the evolution from an early discovertebral lesion - as described in our patient - into a severe destructive discovertebral lesion, by some authors also known as AL. Thus it remains unclear whether and how often an abnormality in the discovertebral junction develops into an AL. More research with a long term follow up of discovertebral lesions is necessary to clarify the evolution of these lesions.

Biomarkers of cardiovascular risk: lipid profile

Patients with AS have approximately a twofold increased mortality rate compared to general population. This is predominantly caused by an increased cardiovascular (CV) risk(12). Inflammation has shown to deteriorate the lipid profile, which is the main risk factor for atherosclerosis. In **Chapter 7**, we noted favourable changes in lipid profile and HDL composition upon TNF blockade. This was reflected by increased HDL-c and Apo A-I levels and an improved Apo B:Apo A-I ratio. Anti-TNF treatment also led to favourable alterations in HDL composition, by diminishing the SAA concentration within the HDL particles, which rendered the lipid profile more atheroprotective.

SAA is an acute-phase reactant, which is synthesized mainly in the liver in response to pro-inflammatory cytokines such as interleukin-1, interleukin-6, and TNF(13), and elevated levels of SAA are associated with increased CV risk(14). Moreover, SAA-rich HDL particles are rapidly cleared from plasma, and thus the increase in SAA during inflammation could also contribute to the decrease of total HDL-c concentrations(15). However, other mechanisms may also play a role in decreased HDL-c levels during inflammation as well. It has been suggested that remodelling HDL through activation of secretory phospholipase A₂ may be an alternate explanation for reduced HDL-c levels during the acute-phase response. In addition, inflammation may convert HDL de novo into a more proatherogenic form by coordinate but inverse transcriptional regulation

of SAA and Apo A-I in the liver(13). This may explain the observed inverse correlation between plasma levels of SAA and Apo A-I, but not between plasma levels of SAA and levels of HDL-c, at baseline. Changes in total cholesterol, HDL-c and Apo A-I levels were significantly inversely associated with changes in levels of disease activity parameters over time, confirming the role of inflammatory activity in lipid profile changes.

Our results highlight the importance of understanding the role of functional characteristics of HDL cholesterol in CV diseases related to chronic inflammatory conditions, such as AS.

We were also interested in studying extraspinal manifestations, such as IBD and conduction disturbances in the heart, as described in **Section III**.

Extraspinal manifestations: Pathophysiological link between AS and IBD

The study in **Chapter 8** reports on the prevalence of serological markers associated with IBD (pANCA, ASCA and OmpC antibodies) in AS patients. For a proper evaluation, three groups of patients with chronic inflammatory diseases were included in this study: one with AS, one with IBD, and one with patients with concurrently AS and IBD.

All determined serological markers were frequently observed in AS patients: pANCA, ASCA IgA, and ASCA IgG antibodies in 21%, 19% and 8% of 52 AS patients, respectively. Furthermore, we demonstrated for the first time that OmpC antibodies are highly prevalent in AS patients (19%). These markers, notably ASCA and OmpC antibodies, rarely occur in healthy controls(28). pANCA was statistically significantly more often present in AS patients with concurrent UC than in AS alone with an OR of 8.2 (95%CI 1.2-55.6). Thus, pANCA might be a valuable tool to screen AS patients with abdominal complaints: if pANCA is present an endoscopy is indicated.

The involvement of the gastrointestinal tract in AS can be interpreted in three different ways: as an aberrant immune response following gastrointestinal infection, as part of an inflammatory disease sharing a common genetic background(29;30) or as a result of intestinal leakage due to treatment with NSAIDs(31).

We have demonstrated that the presence of IBD-associated markers in AS patients is indicative that AS and IBD share a similar pathophysiological origin. These findings apply to AS patients with and without proven IBD since serum markers were also found in AS patients without (symptoms of) IBD. Prospective follow-up of AS patients with positive IBD serology markers in comparison with seronegative patients might shed new light on this discussion and might contribute to the decision whether or not to perform ileocolonoscopy in symptomatic patients and which TNF blocking agents might be most effective, as some (e.g. etanercept) seem to be ineffective in colitis.

Extraspinal manifestations: Conduction disturbances in the heart

Previous literature has revealed that AS patients have an increased risk of conduction disturbances (CD) which is mainly associated with HLA-B27 antigen(32). These studies were mainly based on hospitalized AS patients with a long disease duration and therefore a prospective study was started in our out-patients population of 131 cases (**Chapter 9**). A first-degree AV-block was found in 6 of our AS patients. One patient suffered from a complete right bundle branch block and 1 patient had a left anterior hemiblock. A prolonged QRS-interval (pQRS >100ms) was observed in 38 patients, including those with a complete or incomplete bundle branch block. Age, disease duration and body mass index were significantly associated with PR-interval, and male gender, disease duration, and BASMI with QRS-interval. In the multivariate analyses,

disease duration remained independently associated with both the PR- and QRS-interval.

To conclude, intraventricular CD are highly prevalent in AS, particularly in patients with longstanding disease. Further research is needed to determine whether intraventricular CD may contribute to increased CV risks and long-term cardiovascular mortality in AS.

Future goals

Our main goal is to detect and treat AS, and its complications, at an early stage in order to prevent damage. One of the trials that should be performed is to test the efficacy of very early treatment with anti-TNF, even before abnormalities of AS are visible on radiographs. The goals of such a trial would be to prevent damage and to stop progression of the disease. At this moment we have started such a placebo-controlled trial with etanercept (PREVAS study) at VUmc. Concerning extraspinal manifestations, it is interesting to see whether AS patients with serological markers of IBD will develop manifest IBD in time or not. Particularly AS patients with pANCA and gastrointestinal complaints probably have a higher risk of developing ulcerative colitis. Cardiovascular risks, such as conduction disorders and increased risk of atherosclerosis, can be determined by performing an electrocardiogram and assessment of lipid profile in daily practice, and cardiovascular risk management should be considered. Lowering inflammatory activity by optimum use of TNF blocking agents can be supported by development of reliable biomarkers of disease activity and damage. Further research is needed whether serum trough levels of anti-TNF can be used for clinical decision making and adjustment of the anti-TNF dose. It is possible that non-responsive AS patients require a higher dose, but it is also possible that a lower dose suffices in responsive AS patients. This would lead to a considerable cost reduction in the future. New research has to be done to see

whether concomitant immunosuppressive medication can prevent antibody formation against anti-TNF, which is a significant problem in AS. To conclude, with the introduction of anti-TNF, future perspectives of AS patients have improved dramatically and future studies should aim on refinement of this treatment for individual patients.

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11

Nederlandse Samenvatting

Nieuwe inzichten in de behandeling van de ziekte
van Bechterew met anti-TNF medicatie.

NEDERLANDSE SAMENVATTING

Nieuwe inzichten in de behandeling van de ziekte van Bechterew met anti-TNF medicatie.

Achtergrond

De ziekte van Bechterew is een chronische ontstekingsziekte van de wervelkolom en heiligbeen (sacroiliacaal) gewrichten waarbij tevens gewrichts- en peesontstekingen elders kunnen optreden. Naast het bewegingsapparaat kunnen ook organen aangedaan zijn zoals de ogen (regenboogvliesontsteking (uveitis anterior)), de huid (psoriasis), de darmen (de ziekte van Crohn en colitis ulcerosa), het hart, de longen en soms de nieren. De ziekte leidt tot klachten van rugpijn en stijfheid, die met name 's nachts optreden en verminderen door beweging. In de loop van de tijd kan de ontsteking leiden tot het vastgroeien van de rug (ankylose), verandering in een kromme stand (kyfose), en schade aan de gewrichten toebrengen. In Nederland lijden meer dan 40.000 patiënten aan de ziekte van Bechterew.

De ziekteactiviteit kan onder andere worden gemeten met behulp van een vragenlijst, de BASDAI, die bestaat uit 6 vragen (Appendix).

Tot voor kort bestond de behandeling uit oefentherapie en ontstekingsremmende pijnstillers. Sinds enige jaren is de behandeling met anti-TNF medicatie (gericht tegen het ontstekings eiwit TNF) mogelijk die bij 60% van de patiënten een sterke afname van de ziekteactiviteit geeft. Ten tijde van het schrijven van dit proefschrift waren er 3 middelen beschikbaar, te weten infliximab, adalimumab en etanercept. Infliximab en adalimumab zijn monoklonale antilichamen, waarbij infliximab deels muizeneiwit en adalimumab gehumaniseerd eiwit bevat. Etanercept is een fusie eiwit tussen 2 TNF receptoren. Infliximab wordt 1x/6 weken intraveneus toegediend en

adalimumab en etanercept subcutaan, respectievelijk 2-wekelijks en wekelijks. De meest voorkomende bijwerkingen zijn injectieplaatsreacties en verhoogde gevoeligheid voor infecties.

Sectie I: Immunogeniciteit

In **Hoofdstuk 2 en 3** is onderzocht waarom 40% van de Bechterew patiënten geen baat heeft bij anti-TNF medicatie en waarom bij sommige patiënten de werkzaamheid in de loop van de tijd afneemt.

Patiënten met een goede klinische respons bleken hoge dalspiegels te hebben. Daarnaast bleek er een sterk verband te zijn tussen het niet werken van infliximab of adalimumab en antistofvorming tegen deze middelen. Deze antistoffen werken neutraliserend en zorgen door complexvorming met het medicijn voor een versnelde klaring uit de circulatie. Antistoffen tegen infliximab kunnen uiteindelijk ook een allergische reactie veroorzaken. Voor etanercept hebben wij het verband tussen antistofvorming tegen etanercept en non-respons niet aan kunnen tonen (**Hoofdstuk 4**). Dit zou kunnen komen door de andere structuur van etanercept of doordat het middel vaker wordt toegediend, en daardoor stabielere serumspiegels geeft. Het is ook mogelijk dat er een dosisrespons effect is, maar dit is nog niet onderzocht.

Ineffectiviteit van anti-TNF zou mogelijk kunnen worden tegengegaan door ophoging van de dosis of door voorkoming van antistofvorming door gelijktijdige toediening van immunosuppressiva, zoals methotrexaat of corticosteroiden. Deze gelijktijdige behandeling wordt al toegepast bij patiënten met reumatoïde artritis en met de ziekte van Crohn. Bij deze patiënten lijken inderdaad minder vaak antistoffen gevormd te worden. Naar de rol van gelijktijdige toediening van immunosuppressiva bij anti-TNF voor de ziekte van Bechterew moet nog nader onderzoek worden verricht.

Een andere reden voor ineffectiviteit kan zijn dat de ziekteactiviteit, tot voor kort gemeten met de BASDAI, door de patiënt als ‘hoog’ gescoord wordt door schade die in de loop der jaren door de ontstekingen ontstaan is, terwijl er op dat moment geen hoge ontstekingsactiviteit in het lichaam aanwezig is.

Daarom zijn wij op zoek gegaan naar andere biomarkers voor ziekteactiviteit.

Sectie II: Biomarkers

Biomarkers van ziekteactiviteit

In **Hoofdstuk 5.1**, bleken de ontstekingsfactoren in het bloed zoals de bloedbezinking, maar met name andere acute fase eiwitten zoals C-reactief proteïne (CRP) en serum amyloid A (SAA) geschikt voor het meten van de ziekteactiviteit bij de ziekte van Bechterew. Deze waarden daalden significant na behandeling met infliximab of etanercept. Ook bleek dat patiënten die voor behandeling verhoogde waarden van CRP en SAA hadden vaker goed te reageren op behandeling met antiTNF.

In 2009 werd een nieuwe ziekteactiviteitsscore ontwikkeld: de ASDAS. Deze score is een combinatie tussen vragen over rugpijn, ochtendstijfheid, algemene beoordeling, pijn of zwelling in de gewrichten en de bezinking of het CRP. Wij vinden dat de bezinking minder geschikt is voor het gebruik in de ASDAS door de lange halfwaardetijd in vergelijking tot CRP. In de praktijk kunnen we deze acute fase markers niet altijd gebruiken omdat niet alle Bechterew patiënten reageren met verhoogde ontstekingswaarden in het bloed. In ons onderzoek had 68% van de patiënten met een hoge ziekteactiviteit ook verhoogde ontstekingswaarden. Dit lijkt tevens samen te hangen met genetische aanleg. Uit het onderzoek in **Hoofdstuk 5.2** blijkt dat de CRP spiegel beïnvloed wordt door genetische varianten van het *CRP*-gen, de SNP's (common single nucleotide polymorfismen) en de samenstelling van de genenparen: de haplotypen. SNP

rs3091244 genotypes en het haplotype gelabeld A bleken geassocieerd te zijn met hoge CRP spiegels, onafhankelijk van de ziekteactiviteit (gemeten met de BASDAI) of andere invloeden.

Hoofdstuk 6 beschrijft het voorkomen van typische gelokaliseerde laesies van de wervel en tussenwervelschijf bij Bechterew patiënten: de Andersson laesies. Deze laesies ontstaan waarschijnlijk door een samenspel van schade door ontsteking en mechanische factoren. Uiteindelijk kan er pseudoartrose ontstaan, met name als de wervelkolom vast gegroeid (geankyloseerd) is. Meestal kan de diagnose gesteld worden aan de hand van röntgenonderzoek, maar CT of MRI kunnen extra informatie bieden. De behandeling is chirurgisch en bestaat uit het vastzetten van de wervels en correctie van de kromming (kyfose) van de wervelkolom.

In ons Bechterew cohort werd slechts 1 Andersson laesie gezien, maar deze was alleen zichtbaar op de MRI en niet op de röntgenfoto van de wervelkolom. Het is belangrijk deze laesies tijdig op te sporen, omdat met name de geankyloseerde wervelkolom kwetsbaar is en vaak ook een slechte botkwaliteit heeft (osteoporotisch). Een klein ongeluk kan hierdoor al tot schade en mogelijk neurologische uitval leiden.

Biomarkers van cardiovasculair risico: lipidenprofiel

Bechterew patiënten hebben een verdubbelde mortaliteit ten opzichte van de gehele populatie. Dat komt voornamelijk door een verhoogd risico op hart- en vaatziekten. Het is aangetoond dat ontsteking een slechter lipidenprofiel geeft, hetgeen een hoger risico op aderverkalking (atherosclerose) geeft.

In **Hoofdstuk 7** is te zien dat het lipidenprofiel en de HDL samenstelling van Bechterew patiënten verbeterde na behandeling met anti-TNF. Dit was te zien aan verhoogde HDL-c en Apo A-I spiegels en een betere Apo B:Apo A-I ratio.

Ook leidde de behandeling tot een lagere SAA concentratie in de HDL deeltjes wat een beschermend effect heeft op het ontstaan van atherosclerose. De stijging van totaal cholesterol, HDL-c en Apo A-I hing significant samen met een daling van de ziekteactiviteit.

Sectie III: extraspinale manifestaties

Pathofysiologisch verband tussen Bechterew en inflammatoire darmziekten

In **Hoofdstuk 8** wordt het voorkomen van serologische markers vergeleken bij patiënten met Bechterew, patiënten met inflammatoire darmziekten, en patiënten met beide aandoeningen. pANCA, ASCA IgA en IgG, en OmpC antilichamen zijn geassocieerd met inflammatoire darmziekten. Deze markers kwamen ook voor bij Bechterew patiënten in respectievelijk 21%, 19%, 8% en 19% van de gevallen. Deze markers komen bij gezonde mensen zelden voor.

pANCA kwam significant vaker voor bij Bechterew patiënten met colitis ulcerosa dan bij patiënten zonder deze ziekte met een odds ratio van 8,2 (95% betrouwbaarheidsinterval 1,2-55,6). Daarom kan pANCA een hulpmiddel zijn om colitis ulcerosa te detecteren bij een Bechterew patiënt met darmklachten. Ook bij Bechterew patiënten zonder darmklachten werden deze markers aangetroffen.

Een verklaring voor het voorkomen van deze markers kan liggen in een afwijkende immuunrespons na een darminfectie, een gemeenschappelijke genetische achtergrond, of als gevolg van darmlekkage door behandeling met ontstekingsremmende pijnstillers. Het lijkt er op dat de ziekte van Bechterew en inflammatoire darmziekten een gemeenschappelijke pathofysiologische origine hebben.

Prospectief onderzoek moet in de eerste plaats uitwijzen of de Bechterew patiënten met markers (die met IBD geassocieerd zijn) een inflammatoire darmziekte ontwikkelen en, in de tweede plaats, bij wie van hen er een ileocolonoscopie verricht moet worden.

Geleidingsstoornissen van het hart

Hoofdstuk 9 beschrijft het verhoogd voorkomen van geleidingsstoornissen in het hart bij 131 Bechterew patiënten met lange ziekte duur.

Bij 6 van de patiënten werd op het electrocardiogram (ECG) een 1^e graads AV blok gevonden, 1 patiënt had een compleet rechter bundeltakblok en 1 patiënt had een linker anterior hemiblok. Bij 38 van de patiënten werd een verlengd QRS interval (>100ms) gevonden. In de multivariate analyse was de ziekte duur geassocieerd met het PR en QRS interval. Het is nog onduidelijk of deze geleidingsstoornissen bijdragen aan de verhoogde cardiovasculaire mortaliteit bij Bechterew patiënten.

Toekomstige doelen

Het doel is om de ziekte van Bechterew en de complicaties ervan in een zo vroeg mogelijk stadium op te sporen en te behandelen nog voordat de schade is aangericht. Een van de studies die moet worden gedaan is onderzoek van de effectiviteit van zeer vroege anti-TNF behandeling van de ziekte van Bechterew in het stadium voordat de afwijkingen op het röntgenonderzoek te zien zijn. Het doel van deze behandeling is om de ziekte in een zo vroeg mogelijk stadium tot stilstand te brengen (preventie) en mogelijk zelfs te voorkomen. Op dit moment is zo'n placebogecontroleerde preventiestudie met etanercept opgestart in het VUmc (PREVAS).

Follow-up van de patiënten met markers ten opzichte van de patiënten zonder markers die kunnen duiden op IBD kan interessant zijn, omdat deze groep op

den duur mogelijk een hogere kans heeft op het ontwikkelen van manifeste IBD.

De verhoogde kans op cardiale complicaties, zoals het verhoogd voorkomen van geleidingsstoornissen, kan opgemerkt worden door routinematig een ECG te verrichten, het lipidenprofiel te bepalen en afwijkingen zo nodig te behandelen (cardiovasculair risicomanagement).

Bestrijding van ontsteking door het optimaal inzetten van anti-TNF medicatie kan worden ondersteund door de ontwikkeling van goede biomarkers van ziekteactiviteit en schade. Ook moet onderzocht worden wat een therapeutische spiegel van de antiTNF medicatie is. Daarna kan deze spiegel gebruikt worden voor aanpassing van de dosis om een zo goed mogelijke respons en daling van de ontsteking te bereiken. Het is ook mogelijk dat bepaalde patiënten voldoende hebben aan een lagere anti-TNF dosis wat in de toekomst een aanzienlijke kostenbesparing kan opleveren. Omdat antistofvorming tegen infliximab en adalimumab bij patiënten met de ziekte van Bechterew vaker voorkomt, moeten wij onderzoeken of comedatie met immunosuppressiva kan helpen dit te voorkomen, zodat meer patiënten langer baat hebben bij deze medicatie.

Tot slot kan gezegd worden dat het toekomstperspectief voor veel patiënten met de ziekte van Bechterew er door de komst van anti-TNF medicatie aanzienlijk op vooruit is gegaan. De komende studies zullen zich vooral moeten richten op verfijning van deze behandeling voor de individuele patiënt.

Appendix

Questionnaires and BASMII

Abbreviations List

Dankwoord

Curriculum Vitae

QUESTIONNAIRES AND BASMI

BAS-G (numerical rating scale)

1. Kruis een blokje aan, om aan te geven in welke mate uw ziekte van invloed was op uw algeheel welbevinden gedurende de afgelopen week.

0	1	2	3	4	5	6	7	8	9	10
---	---	---	---	---	---	---	---	---	---	----

geen zeer veel

2. Kruis een blokje aan, om aan te geven in welke mate uw ziekte van invloed was op uw algeheel welbevinden, de afgelopen zes maanden.

0	1	2	3	4	5	6	7	8	9	10
---	---	---	---	---	---	---	---	---	---	----

geen zeer veel

Pain (numerical rating scale)

Kruis s.v.p. het blokje aan dat uw antwoord het best weergeeft, b.v.



1. Hoeveel rugpijn had u 's nachts gedurende de afgelopen week?

0	1	2	3	4	5	6	7	8	9	10
---	---	---	---	---	---	---	---	---	---	----

geen pijn ondraaglijke pijn

2. Hoe erg was de pijn van de rug gemiddeld de afgelopen week?

0	1	2	3	4	5	6	7	8	9	10
---	---	---	---	---	---	---	---	---	---	----

geen pijn ondraaglijke pijn

Patient global disease activity (numerical rating scale)

Hoe actief was uw ziekte gemiddeld de afgelopen week?

0	1	2	3	4	5	6	7	8	9	10
---	---	---	---	---	---	---	---	---	---	----

niet actief heel erg actief

BASFI (numerical rating scale)

Kruis een blokje aan om aan te geven in welke mate u in staat was de onderstaande activiteiten uit te voeren **gedurende de laatste week** b.v.



1. Uw kousen of panty's aantrekken zonder hulp of hulpmiddelen (b.v. kousentrekker)

0	1	2	3	4	5	6	7	8	9	10
---	---	---	---	---	---	---	---	---	---	----

gemakkelijk onmogelijk

2. Vanuit de heup naar voren buigen om zonder hulp een pen van de grond te rapen.

0	1	2	3	4	5	6	7	8	9	10
---	---	---	---	---	---	---	---	---	---	----

gemakkelijk onmogelijk

3. Aan een hoge plank kunnen zonder hulp of hulpmiddelen (b.v. helpende hand).

0	1	2	3	4	5	6	7	8	9	10
---	---	---	---	---	---	---	---	---	---	----

gemakkelijk onmogelijk

4. Rechtkomen uit een eetkamerstoel zonder armleuning, zonder uw handen of andere hulp te gebruiken.

0	1	2	3	4	5	6	7	8	9	10
---	---	---	---	---	---	---	---	---	---	----

gemakkelijk onmogelijk

5. Zonder hulp van de grond komen als u op uw rug ligt.

0	1	2	3	4	5	6	7	8	9	10
---	---	---	---	---	---	---	---	---	---	----

gemakkelijk onmogelijk

6. Gedurende 10 minuten zonder steun blijven staan zonder ongemakken.

0	1	2	3	4	5	6	7	8	9	10
---	---	---	---	---	---	---	---	---	---	----

gemakkelijk onmogelijk

7. Een trap van 12-15 treden opgaan zonder een leuning of wandelstok of dergelijke te gebruiken.
Eén voet op elke trede.

0	1	2	3	4	5	6	7	8	9	10
gemakkelijk					onmogelijk					

8. Over uw schouder kijken zonder uw lichaam te draaien.

0	1	2	3	4	5	6	7	8	9	10
gemakkelijk					onmogelijk					

9. Fysiek zware activiteiten uitvoeren (b.v. fysiotherapie oefeningen, tuinieren of sport).

0	1	2	3	4	5	6	7	8	9	10
gemakkelijk					onmogelijk					

10. Een volledige dagtaak thuis of op het werk uitvoeren.

0	1	2	3	4	5	6	7	8	9	10
gemakkelijk					onmogelijk					

BASMI

	0	Score 1	2
Tragus-muur afstand	<15 cm	15-30 cm	>30 cm
Schober	>4 cm	2-4 cm	<2 cm
Cervicale rotatie	>70°	10-70°	<10°
Lumbale lateroflexie	>10 cm	5-10cm	<5 cm
Intermalleolair afstand	>100 cm	70-100 cm	<70 cm

ABBREVIATIONS LIST

AL	Andersson lesion
ANA	antinuclear antibodies
Anti-TNF	TNF blocking medication
apoA-1	apolipoprotein A1
apoB	apolipoprotein B
AS	ankylosing spondylitis
ASAS	assessments in ankylosing spondylitis
ASCA	antibodies to the cell-wall mannan of <i>Saccharomyces cerevisiae</i>
ASDAS	ankylosing spondylitis disease activity score
AU	arbitrary units
BASDAI	Bath ankylosing spondylitis disease activity index
BASFI	Bath ankylosing spondylitis disease functional index
BASG	Bath ankylosing spondylitis disease global
BASMI	Bath ankylosing spondylitis disease metrology index
BMI	body mass index
C	cervical
CD	Crohn's disease (chapter 9)
CD	conduction disturbance (chapter 10)
CI	confidence interval
CRP	C-reactive protein
CV	cardiovascular
DDD	degenerative disk disease
DDL	destructive discovertebral lesion
DMARDs	disease modifying anti-rheumatic drugs
e.g.	exempli gratia/for example
ELISA	enzyme-linked immunosorbent assay
ESR	erythrocyte sedimentation rate
Gd-DTPA	gadolinium diethylenetriamine pentaacetic acid
GEE	generalized estimating equations
GI	gastrointestinal
HDL-c	high density lipoprotein cholesterol
HLA-B27	human leucocyte antigen B27
hsCRP	high sensitive CRP

IBD	inflammatory bowel disease
i.e.	id est/that means
Ig	immunoglobulin
IQR	interquartile range
IBI	Jan van Breemen Institute
kg	kilogram
L	lumbar
LDL-c	low density lipoprotein cholesterol
LS	lumbosacral
MRI	magnetic resonance imaging
MTX	methotrexate
N	number
n.k.	not known
NA	not applicable
NR	non-responder
NRS	numeric rating scale
NSAID	nonsteroidal anti-inflammatory drug
OmpC	antibodies to porin protein C of Escherichia coli
OR	odds ratio
pANCA	perinuclear antineutrophil cytoplasmic antibodies
pPR	prolonged PR interval
pQRS	prolonged QRS interval
PPM	phosphopeptidomannan
R	responder
RA	rheumatoid arthritis
RIA	radioimmunoassay
ROC	receiver operating characteristic
SAA	serum amyloid A protein
SD	standard deviation
SELDI-TOF	surface-enhanced laser desorption/ionization time-of-flight
SNPs	single-nucleotide polymorphisms
SpA	spondylarthropathy
SPSS	statistical package for the social sciences
STIR	short tau inversion recovery

T	thoracic
TC	total cholesterol
TL	thoracolumbar
TNF	tumor necrosis factor
t-test	students t-test
UC	ulcerative colitis
VAS	visual analogue scale
VU	vertebral unit
VUmc	VU University medical center
Yrs	years

DANKWOORD

Graag wil ik iedereen bedanken die zijn steentje heeft bijgedragen bij de totstandkoming van dit proefschrift! Een aantal mensen wil ik speciaal bedanken.

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Hugo, bedankt voor je liefde. Bij jou ben ik thuis. Met Fenna is ons geluk gegroeid!

CURRICULUM VITAE

The author of this thesis was born in The Hague on the 16th of February 1978. After finishing her secondary school in 1996 at the Dalton school in The Hague she studied medicine at the Leiden University. In 1998, she performed physical anthropological research on ten skeletons from an excavation in Breda, under the supervision of professor Maat. In 2001, she received her master's degree and in 2002, after the internships, the last one being an internship internal medicine in Paramaribo, Suriname, she received her medical doctor's degree. After working as an internal medicine resident in The Hague, she started working on her thesis at the rheumatology department of VU University medical center in 2004. Then, she became interested in becoming a rheumatologist. During this period she also studied clinical epidemiology and she received her master's degree in 2008. Starting October 2010 until March 2012, she worked as a resident internal medicine at the Spaarne hospital in Hoofddorp, and subsequently VUmc. At the moment she works at Reade as part of her formal training to become a rheumatologist, which she will complete in 2015 at VUmc.

De auteur van dit proefschrift werd op 16 februari 1978 geboren te 's-Gravenhage. Na het behalen van haar VWO diploma aan de Daltonschool te 's-Gravenhage startte zij haar studie Geneeskunde aan de Universiteit Leiden. In 1998 deed zij fysisch antropologisch onderzoek op tien skeletten uit een opgraving in Breda onder supervisie van professor Maat. In 2001 behaalde zij haar doctoraalexamen en na het doorlopen van de coschappen, met als laatste een coschap interne geneeskunde in Paramaribo te Suriname, behaalde zij in 2002 haar artsexamen. Hierna werkte zij enige tijd als arts-assistent interne geneeskunde in 's-Gravenhage, waarna zij in 2004 op de afdeling reumatologie aan het VU medisch centrum te Amsterdam aan haar promotieonderzoek begon te werken. Hier werd de interesse voor het werken als reumatoloog gewekt. Ook volgde zij tijdens deze periode de masteropleiding epidemiologie waarvan in 2008 haar bul ontving. Vanaf oktober 2010 tot maart 2012 werkte zij achtereenvolgens als arts-assistent interne geneeskunde in het Spaarne ziekenhuis te Hoofddorp en het VUmc. Op dit moment werkt zij in Reade in het kader van de opleiding tot reumatoloog, die zij in 2015 aan het VUmc zal afronden.